# Laboratory Procedure Manual

*Analytes:* **Aromatic Amines**

*Matrix:* **Urine**

*Method:* **GC Tandem Mass Spectrometry**

*Method No:* **2020**

*Revised:*

As performed by:

**Tobacco and Volatile Branch**
**Division of Laboratory Sciences**
**National Center for Environmental Health**

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**Important Information for Users**

The Centers for Disease Control and Prevention (CDC) periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporate

# Division of Laboratory Sciences

# Laboratory Protocol

**Analytes:** *o*-toluidine, 2,6-dimethylamine, *o*-anisidine, 1-aminonaphthalene, 2-aminonaphthalene, 4-aminobiphynyl

**Matrix:** Urine

**Method:** Gas Chromatography Tandem Mass Spectrometry (GC–MS/MS)

**Method code:** 2020.02

**Branch:** Tobacco and Volatile Branch

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Date current version of method first used in lab: _____ (Date)

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Reviewed: _____ (Signature) _____ (Date)

# Procedure Change Log

**Procedure: Aromatic amines in Urine**
**DLS Method Code: 2020.02**

| Date | Changes Made | By | Rev'd By (Initials) | Date Rev'd |
|---|---|---|---|---|
| 08/13/2016 | **Section 2.h:** Disposal of wastes-10% bleach solution was replaced by Lysol® I.C Quaternary Disinfectant Cleaner solution<br>**Section 6.a:** Handling information for solvents and reagents- Acetic Anhydride is no longer used. | sm | | 08/13/2017 |
| 06/20/2016 | **Section 8.d.1:** Analyzing and storing GC/MS-MS data- Indigo ASCENT™ was used to process chromatographic data and quantification in place of Agilent MassHunter™ software. | sm | | 06/20/2016 |
| 08/22/2016 | **Section 8.d.1:** Analyzing and storing GC/MS-MS data- Repeat manager database was added | ra | | 08/22/2017 |
| 08/31/2016 | **Appendix A.A.1:** Native standard<br>• Table A1: Stock B was added (OTOL only).<br>• Table A2: Standard 13-17 were added (OTOL only) to extend OTOL dynamic range. | ts | | 08/31/2016 |
| 04/06/2017 | **Section 6.g:** Instrumentation<br>• GC column 1 changed from DB-17MS to DB-FFAP.<br>• Post column is added for backflush (listed as column 1 in Instrument Operation Parameter).<br>• GC temperature gradient- final temperature changed from 280ºC to 240ºC due to new GC column 1.<br>• GC temperature gradient- post run hold for 5 minutes at 240ºC during backflush. | sm | | 04/06/2017 |
| 12/16/2016 | **Appendix A.B.1:** Quality control materials<br>• Table B1: QCLow_2016 and QCHigh_2016 were added | ts | | 12/16/2016 |
| 05/18/2017 | Method cross validation for column DB-17MS and DB-FFAP and all changed parameters that were made to GC method (for sample in-reinjections). | sm | | 05/18/2017 |
| 11/11/2017 | Completed method re-validation<br>**Appendix B:** all method validation data were update | ts | | 11/16/2017 |
| 11/29/2017 | **Appendix C:**<br>Method performance documentations are added. | ts | | 12/29/2017 |
| 02/16/2018 | **Appendix B:** updated LoDs | ts | | 02/20/2018 |
| 04/18/2018 | **Appendix B:** updated LoDs, table B4 | ts | | 06/20/2018 |
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## Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

| DATA FILE NAME | VARIABLE NAME | ANALYTE DESCRIPTION |
|---|---|---|
| **AA_H & AAS_H** | URX1NP | 1-Aminonaphthalene, urine (pg/mL) |
| | URX2NP | 2-Aminonaphthalene, urine (pg/mL) |
| | URX4BP | 4-Aminobiphenyl, urine (pg/mL) |
| | URXANS | o-Anisidine, urine (pg/mL) |
| | URXDMN | 2,6-Dimethylaniline, urine (pg/mL) |
| | URXOTD | o-Toluidine, urine (pg/mL) |

# 1. CLINICAL RELEVANCE AND SUMMARY OF TEST PRINCIPLE

## A. *Analytes*

o-toluidine (OTOL), C7H9N; Mol Wt 107.15
2,6-dimethylaniline (26DM), C8H11N, Mol Wt 121.18
o-anisidine (OANS), C7H9NO, Mol Wt 123.15
1-aminonaphthalene (1-AMN), C10H9N, Mol Wt 143.19
2-aminonaphthalene (2-AMN), C10H9N; Mol Wt 143.19
4-Aminobiphenyl (4-ABP), C12H11N; Mol Wt 169.22

## B. *Clinical Relevance*

o-toluidine (OTOL), o-anisidine (OANS), 2-6-dimethylaniline (26DM), 2-aminonaphthalene (2-AMN), and 4-aminobiphenyl (4-ABP) are classified as carcinogens or possible carcinogens (1-6). They are present in mainstream and sidestream tobacco smoke, with the latter containing up to thirty times as much 4-ABP as mainstream smoke (7-8). Aromatic amines are metabolized mainly in the liver where they are N-glucuronidated, N-acetylated, or form N-hydroxyarylamine via oxidation. Aromatic amines are believed to exert their carcinogenic effect by reaction of the n-hydroxylamine metabolite with DNA in the target organ (e.g. bladder) (7-8). Free or conjugated forms (N-acetylated or glucuronidated) are excreted in urine directly from the bladder. Consequently, urinary concentrations of aromatic amines are effective surrogate measures of the carcinogenic metabolite at the target tissues (bladder, liver, kidney, pancreas, spleen, thyroid, etc.).

## C. *Assay Principle*

OTOL, OANS, 26DM, 1-AMN, 2-AMN, and 4-ABP are quantified by an isotope-dilution gas chromatographic, tandem mass spectrometric method (ID GC-MS/MS). Urine samples are collected and stored at approximately -70±10°C. 13C and 2H internal standards are added, and the samples are hydrolyzed, cleaned up, and extracted on support liquid extraction (SLE) cartridges. The analytes are then derivatized to form pentafluoropropionamides, and analyzed by GC/MS/MS, using multiple reaction monitoring (MRM). The analyte concentrations are derived from the ratio of the integrated peaks of native to labeled ions by comparison to a standard curve.

## D. *Special Precaution*

Because of the sensitive nature of these assays and the off-gassing of the target analytes by smokers, all analysts performing this method must be nonsmokers, and measurements must be performed in a smoke-free environment.

# 2. SAFETY PRECAUTIONS

## A. *Reagent Toxicity or Carcinogenicity*

Many aromatic amines (AAs) are carcinogenic. Care should be taken to avoid inhalation or dermal exposure. Use a chemical fume hood when working with AAs. Appropriate use of personal protection including lab coats, gloves, and safety glasses are required when preparing or handling neat materials, standard solutions, extraction solutions, or collected samples.

## B. *Radioactive Hazards*

None.

## C. *Microbiological Hazards*

This assay involves human urine samples. Universal precautions must be followed. Analysts working directly with the specimens must use proper technique and avoid any direct contact with the samples. Lab coats, gloves, and safety glasses (as required) should be worn while handling the specimens.

## D. *Mechanical Hazards*

There are no unusual mechanical hazards associated with this method. Analysts should know and follow the manufacturer's recommendations concerning the safe handling of instruments and other equipment. High voltages are found within certain areas of the mass spectrometer and care must be taken when working in those areas.

## E. *Protective Equipment*

Standard chemical laboratory personal safety equipment is required including lab coats, gloves, and safety glasses.

## F. *Training*

Training for sample preparation, sample handling, and equipment operation is required.

## G. *Personal Hygiene*

Follow standard precaution and comply with all established laboratory safety practices. Care should be taken when handling chemicals to avoid inhalation or dermal exposure. Lab coats, gloves, and safety glasses should be worn at all times handling standards or samples.

### H. *Disposal of Wastes*

Dispose all waste materials in compliance with laboratory, federal, state, and local regulations. Solvents and reagents should always be disposed of in an appropriate container that has been clearly marked for waste products and temporarily stored in a chemical fume hood. Place disposable laboratory supplies such as vials, pipette tips, syringe, etc. that directly contact AAs or samples in a biohazard autoclave bag or similar approved storage container. Unshielded needles, glass pipets and disposable syringes with attached needles must be placed in a sharps container and autoclaved when the container is full. Wipe down all surfaces potentially exposed to biological samples with Lysol® I.C Quaternary Disinfectant Cleaner after each sample preparation. Non-disposable glassware or other equipment that comes into contact with biological samples must be rinsed with bleach before reuse.

## 3. COMPUTERIZATION; DATA-SYSTEM MANAGEMENT

### A. *Software and knowledge requirements*

This method has been validated using an automated sample preparation system - followed by gas chromatography tandem mass spectrometry. The Agilent GC Triple Quad 7000C is controlled by MassHunter™ software. Indigo ASCENT™ is utilized in chromatographic and MS quantitation analysis. Proficiency is required for the analytical software package of automation system, GC, and mass spectrometer used in the analysis. Further, statistical analysis of results requires proficiency in a standard statistical analysis software package. The Statistical Analysis System (SAS Institute, Cary, NC) is one such.

### B. *Sample information*

Typically samples are analyzed in runs of 32-64 samples -including one water blank, one quality control (QC) low, one QC high and unknowns. Each run is identified as “AYYMMDDxxxx” (YearYearMonthMonthDateDateAnalyst’userID, e.g. A160211leo9). Each sequence file contains such information as Run ID, sample ID, sample file name, date of analysis, analyst, and sample volume. The GC/MS/MS relative response data are transferred electronically into the database for each sample and associated calibrators, QCs, and blanks.

### C. *Data maintenance*

Check data entered into the database for transcription or transmission errors. Routinely back-up the database on a weekly basis or as needed (software update, etc.).

### D. *Information security*

Information security is provided at multiple levels. The data systems used in this work are accessed via computers that require individual login and passwords and that default to locked conditions during extended periods of nonuse. Sensitive portions of custom software are protected with additional password requirements. In addition, on the Chamblee campus of CDC, all systems and equipment have restricted access wth security personnel approving all entry. Furthermore, the individual laboratory building has multiple levels of controlled access including the requirement for key cards to access the building itself, and also the individual floors where the equipment is located. Confidentiality of the results is protected by use of blind coded ID numbers (no clinical specimen are ever labeled with personal identifiers).

## 4. COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

### A. *Special requirements*

There are no special requirements such as fasting or adherence to special diets for this assay.

### B. *Sample collection*

The specimen for these analyses is human urine. Based on the relatively short physiological half-lives of these analytes, urine samples integrating over longer time periods are preferred over spot urine samples.

### C. *Sample handling*

Specimens should be frozen prior to shipment, must be sent and received frozen where they will be stored at -70±10°C until analysis. All samples are vortex thoroughly prior to preparation (more details in sample preparation section).

### D. *Sample quantity*

The sample size is 2.00 ml of urine. This sample volume is required to quantify the analyte concentrations listed for the Limits of Detection in Section 9a. However, smokers have much higher concentrations of these analytes, so detectable concentrations can likely be measured in a reduced volume sample collected from a smoker (1 ml).

### E. *Sample rejection criteria*

Criteria for defining a sample as unacceptable include (1) use of improper collection materials or techniques leading to possible background

contamination; and (2) sample volume below 1 ml.

### F. *Long-term stability*

The OTOL, OANS, 26DM, 1-AMN, 2-AMN, and 4-ABP are stable in urine samples stored in glass or cryogenic polypropylene tubes at low temperatures, -70±10ºC for at least 2-10 years.

## 5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

## 6. PREPARATION OF REAGENTS, CALIBRATORS (STANDARDS), CONTROLS, AND ALL OTHER MATERIALS; EQUIPMENT AND INSTRUMENTATION

Note: Class A glassware such as pipets and volumetric flasks are used unless otherwise stated.

### A. *Handling information for solvents and reagents*

(1) o-toluidine, 2,6-dimethylaniline, o-anisidine, 1-aminonaphthalene, 2-aminonaphthalene, 4-aminobiphenyl. These chemicals are known or suspected carcinogens, and suitable protective clothing, gloves and eye/face protection must be used. It can be dangerous if inhaled, swallowed or absorbed through the skin, and should only be used in a chemical safety hood. If contact occurs, flush area immediately with copious amounts of water. If inhaled, remove to fresh air or consult a physician.

(2) Sodium hydroxide. This chemical is a caustic base that is corrosive to all tissues. It generates considerable heat when mixed with water or an acid. It is nonflammable but would be harmful if inhaled or swallowed. Protective clothing and safety glasses must be worn while working with this reagent.

(3) Hexane. This is a flammable solvent. The vapor or mist is irritating to the skin, eyes, mucous membranes and upper respiratory tract. Protective clothing and safety glasses should be used. Use chemical fume hood when working with this solvent.

(4) Toluene. This is a flammable liquid and may form explosive vapors. Toluene vapor is heavier than air and may travel some distance to an ignition source. Toluene forms an irritating vapor. As a liquid it is a skin

irritant, and may be absorbed through the skin. Large volumes of toluene should be handled with gloves in a chemical fume hood.

(5) 1.07 M Trimethylamine. Aldrich 98% #T7, 276-1 FW 95.6, stored in a desiccator. Weigh 1.0 gm trimethylamine-HCl at 98% (0.98 gm TMA-HCl) and dissolve in 2.0 ml B&J water, neutralized with 10 M NaOH, approx. 1-3 µl, and extract with 5.0 ml hexane (Burdick & Jackson #216-4 high purity solvent). Transfer 3.0 ml of the hexane extract to a clean 10 ml vial and add 3.0 ml additional hexane. Final concentration is 1.0 M, reagent is stored in refrigerator up to 6 months.

(6) Methylene chloride. This solvent is chemically stable and relatively unreactive. It is not flammable, but the vapor can be irritating to the eyes, nose and throat. Skin or eye contact with the liquid should be avoided. Flush exposed tissue copiously with water if any contact should occur. Evaporation of significant volumes of this solvent must be performed in the Savant evaporator, or in a chemical fume hood.

(7) Ethanol. This is a flammable solvent whose vapors can form ignitable and explosive mixtures with air at normal room temperatures. Exposure to liquid, vapors, fumes, or mist may cause irritation, redness, pain, and possible corneal damage. Prolonged contact may lead to defatting, redness, pain, itching, inflammation, and possible secondary infections. Use appropriate gloves, lab coat, safety glass, and recommended PPE (personal protective equipment) when handling this reagent.

## B. *Stock reagent preparation*

Native standards are weighed and diluted in ethanol to make Stock A. Chemical purity, lot and stock concentrations would be different each time a new stock is made. An example of Stock A is listed below.

| Date 1mg/mL Stock Made | Analyte | Vendor | Chemical Purity (%) | Purity Test | Lot | Analyte Weight (mg) | Stock A Conc.* (mg/mL) |
|---|---|---|---|---|---|---|---|
| 03/31/15 | OTOL | Fluka | 99.5 | GC | 1421704V | 10.720 | 1.0666 |
| 07/28/14 | 26DM | Aldrich | 98.5 | GC | STBB5452V | 7.840 | 0.7722 |
| 07/28/14 | OANS | Fluka | 99.7 | GC | SZBB343XV | 9.630 | 0.9601 |
| 03/31/15 | 1AMN | Fluka | 99.9 | GC | SZB8268XV | 10.100 | 1.0090 |
| 03/31/15 | 2AMN | Sigma | 98.0 | TLC | SLBD0851V | 11.090 | 1.0868 |
| 07/28/14 | 4ABP | Aldrich | 99.0 | TLC | 128K1386V | 10.830 | 1.0722 |

***Made in 10-mL volumetric flask, Stock A in ethanol, further dilution in hexane*

| Date 1mg/mL Made | Analyte | Vendor | Chemical Purity (%) | Isotopic Purity (%) | Purity Test | Lot | Actual Weight (mg) | Stock DA Final Con.* 10 ml (mg/mL) |
|---|---|---|---|---|---|---|---|---|
| 01/29/15 | OTOL-$^{13}C_6$ | MI | 98.0 | 98.0 | Mass Spec | 713 | 2.360 | 0.2267 |
| 07/28/14 | PTOL-$D_9$ | MI | 98.6 | 99.0 | GC | 18 | 9.230 | 0.9010 |
| 07/28/14 | MTOL-$D_9$ | MI | 98.4 | 99.0 | GC | 310 | 9.070 | 0.8836 |
| 07/28/14 | 26DM-$D_6$ | TRC | 98.0 | 95.3 | Mass Spec | 3-MIC-149-1 | 9.000 | 0.8405 |
| 07/28/14 | OANS-$D_7$ | MI | 99.8 | 99.4 | HPLC & NMR | 533 | 10.180 | 1.0099 |
| 01/29/15 | 2ABP-$D_9$ | MI | 99.0 | 99.6 | HPLC | 932 | 10.430 | 1.0284 |
| 07/28/14 | 1AMN-$D_9$ | MI | 98.1 | 99.1 | HPLC & NMR | 145 | 10.790 | 1.0490 |
| 12/21/15 | 2AMN-$D_7$ | CDN | 98.0 | 95.0 | NMR & Mass Spec | Z-330 | 3.170 | 0.3170 |
| 07/28/14 | 3ABP-$D_9$ | TRC | 98.0 | 98.7 | Mass Spec | 12-SDJ-187-1 | 8.700 | 0.8415 |
| 07/28/14 | 4ABP-$D_9$ | CI | 99.0 | 99.6 | HPLC & NMR | I1-9733 | 9.570 | 0.9436 |

**Made in 10-mL volumetric flask, Stock A in ethanol, further dilution in hexane, concentration is adjusted by purity as described in Appendix A.*

Toluene. Burdick & Jackson, ACS/HPLC Grade GC 99.96% purity.
Hexane. Burdick & Jackson, High purity GC 99.63% purity.
Ethanol. Pharmco, ACS Grade GC 99.9% purity.

## C. *Calibrators*

Prepare one complete set of calibration standards (50-100 mL) at one time for use over a period of several years. Prepare labeled $^{13}C$ or $^{2}H$ (D) internal standards at the same time or as needed and then aliquot, seal, and store these solutions at approximately -20ºC±4ºC. The internal standard spiking (ITSD) solution for unknown samples was prepared in ethanol, dispensed in 4.0 ml aliquots; and stored at approximately -20ºC±4ºC. Derivative standards and ISTD spiking solutions were prepared as described below. Analyze at least three calibration curves to confirm acceptable linearity ($R^2 > 0.98$). A standard curve is run with every analytical run/batch. A total of 14 standards were prepared ranging from 0 to 200 pg/µl (details in Appendix A) for all analytes except for OTOL, which has 17 standards and a higher range (1,300 pg/µl)

Derivatization of Hexane Standards. 5 mL of each standard is aliquoted via 5-mL volumetric flask. A known amount of internal standard of labelled-analytes is added, followed by TMA and PFAA (pentafluoropropionic acid anhydride, Pierce #65193M MW 310.0). The standards were capped, vortexed, held at room temperature for 30-40 minutes, transferred to silanized tubes, and dried completely in a Savant Speedvac System. Reconstitution to 5 mL toluene is done by 2x2 mL rinse of the silanized tubes, transferred to 5-mL volumetric flasks, and bring to final volume mark. Final standard solutions are aliquoted to 5x1 mL into high recovery amber vials, capped, labelled, and stored at -20°C±4ºC. Larger volume, up to 25 Ml, can be prepared with volumetric flask.

Details and actual concentrations for each analyte of the currently standard curve are in Appendix A.

**Concentrated Internal Standard Spiking Solution:**

Internal standard (ISTD) spiking solution for unknown sample preparation was made by diluting the DA and DC stock solutions in ethanol. The ITSD spiking solution was dispensed into 4.0 mL aliquots and stored frozen at -20±4$^{o}$C. Appendix A provided the details of how the current ISTD spiking solution was prepared.

## D. *Controls*

(1) **Quality control (QC) materials.**There are two quality control pools for the urinary aromatic amines assay: low and high. The QC pools were prepared in-house from urine collected from non-smokers, filtered with 0.2 µm filters and spiked with standard stock solution containing 6 analytes: *o*-toluidine, 2,6-dimethylaniline, *o*-anisidine, 1-aminonaphthalene, 2-aminonaphthalene, and 4-aminobiphenyl. The pool concentrations were made at approx. 100-125 pg/mL and 400-500 pg/mL, respectively. Details and exact concentrations for each analyte can be found Appendix A. Each pool was mixed well, dispensed (with constant stirring) in 2.4 mL aliquots into 5mL cryovials with screw cap, and stored frozen at -70±10°C. One box of each QC is kept in a convenient freezer for daily analysis.

(2) ***Proficiency testing (PT) materials.*** At this time, there are no external PT programs or certified reference materials for aromatic amines in human urine. Therefore, we developed an in-house PT program administered by a QC officer (more details are in Section 10c).

## E. *Other Material and Supplies*

Materials, supplies and their sources that used during the development and validation are listed below. Materials and supplies for use with this method should be equivalent to those listed if obtained from other sources.

- Pipettes and disposable tips capable of accurately dispensing the following volumes: 50 µl to 200 µl, 1 ml, 5 ml (Hamilton)
- Gas-tight syringes capable of accurately dispensing the following ranges of volumes: 1-100 µL, 5-500 µL, 0.1-2.5 mL (Hamilton)
- High recovery 3.7 mL glass vials (ChemGlass)
- PTFE coated cap (Wheaton)
- Disposable silanized test tubes, 13x100 mm (Fisher Scientific)
- 300 µl insert 12x32 mm amber vials (Wheaton)

- Assorted glassware

## F. *Equipment.*

- Commercial Hamilton STAR automation system
- Commercial tandem mass spectrometer such as the Agilent triple quad G7000C (or comparable)
- Commercial gas chromatography system (GC) such as the Agilent 7968 GC system (or comparable).
- Commercial autosampler system (AS) such as the Agilent G4514A system, GC Sampler 80 (or comparable)
- Commercial Thermo Savant SpeedVac SPD 2010 (vacuum evaporator).
- Digital block heater, VWR block heater

## G. *Instrumentation*

(1) Gas chromatograph

Instrument Operating Parameters

| **Instrument Operation Parameter (Agilent 7890A, 7890B)** | **Setting** |
|---|---|
| Injection port | Pulsed splitless |
| Injection port temperature | 250°C |
| Injection volume | 1 µl |
| Injection Pulse Pressure | 30 psi until 0.35 min |
| Septum Purge Flow | 3 mL/min |
| Purge flow to split vent | 60 mL/min at 0.35 min |
| Column 1 (Agilent J&W, DB-FFAP, 122-3232) | Constant flow 1.68 ± 0.05 mL/min (analytical run); Constant flow -2.20 ± 0.05 mL/min (post-run) |
| Column 2 (Agilent J&W, Inert fused silica, 160-7265-5) | Constant flow 1.85 ± 0.05 mL/min (analytical run); Constant flow 10.5 ± 0.05 mL/min (post-run) |
| Liner (Agilent 5183-4693) | 4mm ID, 900 µL splitless, single taper, glass wool, deactivated |
| MSD Transfer Line | 280°C |
| Oven program (analytical run) | 80°C (hold 2 min); 30°C/min ramp to 180°C; 15°C/min ramp to 240°C |
| Oven program (post- run) | 240°C (hold 5 min) |

## Mass Spectrometer Source/Gas Parameters

| **Instrument Operation Parameter (Agilent 7000C)** | **Setting** |
|---|---|
| Mass spectrometer mode | Positive ion detection |
| Ionization source | Electron impact (EI) |
| Source temperature | 280°C |
| Quad temperature | 150°C |
| Collision gas | Nitrogen at 1.5 mL/min |
| Collision pressure | 35 psi |
| Quench gas | Helium at 2.25 mL/min |
| Detector setting | Gain at 20 (x $10^5$) |

## Multiple Reaction Monitoring Parameters

The ion pairs and compound dependent parameters are listed below.

| Analyte | Precursor ion | Product ion | Collision energy | Dwell time |
|---|---|---|---|---|
| | (*m/z*) | (*m/z*) | (*V*) | (*ms*) |
| OTOL | 253 | 116 | 30 | 55 |
| | 253 | 106 | 25 | 17 |
| | 259 | 122 | 30 | 35 |
| | 259 | 112 | 25 | 35 |
| 26DM | 267 | 148 | 10 | 65 |
| | 267 | 120 | 25 | 65 |
| | 273 | 154 | 15 | 65 |
| | 273 | 126 | 30 | 65 |
| OANS | 269 | 135 | 30 | 35 |
| | 269 | 190 | 15 | 55 |
| | 276 | 139 | 35 | 35 |
| | 276 | 157 | 15 | 35 |
| 1AMN | 289 | 144 | 20 | 45 |
| | 289 | 127 | 30 | 45 |
| | 295 | 150 | 20 | 45 |
| | 296 | 151 | 20 | 45 |
| 2AMN | 289 | 144 | 20 | 45 |
| | 289 | 127 | 30 | 45 |
| | 296 | 151 | 20 | 45 |
| | 295 | 150 | 20 | 45 |
| 4ABP | 315.1 | 170 | 30 | 65 |
| | 315.1 | 141 | 40 | 65 |
| | 324 | 179 | 20 | 65 |
| | 324 | 150 | 45 | 65 |

**Ion units in m/z. **DT = Dwell time units in milliseconds. CE = collision energy (in Volts).*

The Agilent Triple Quad specific operational variables CE and DT refer to collision energy and dwell time.

(2) GC-MS/MS Instrument Control Program

An instrument control program for the Agilent GC/MS Triple Quad created using the MassHunter™ software that incorporates the above parameters is used for data acquisition.

# 7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

## A. *Calibration Curve*

(1) Data Collection

A calibration curve is constructed at the beginning of each study using response factors (i.e., peak area ratio of analyte to labeled internal standard) versus calibration standard concentration of 14-17 calibrators (pg/µl). All 14-17 standard solutions are injected 3 times and subsequently analyzed on the GC/MS-MS to evaluate the linearity response for each analyte. A calibration curve is obtained with each analytical run and after a major service on the instrument (such as oil change, filament replacement, source cleaning, etc.).

(2) Calculation and Evaluation of Curve Statistics

The slope and intercept of the calibration curve are generated using linear regression with 1/x weighting. This analysis can be performed using the instrument's data analysis software or other suitable data analysis software (such as Indigo ASCENT™). The resulting plot should be examined for linearity over the entire calibration range (R-square ≥ 0.98). Determine the slope and intercept of the calibration curve by linear least squares fit. Any deviations from this procedure (e.g., using a quadratic fit) must have a valid scientific justification and be approved by a supervisor.

## B. *Usage of Curve*

The calibration range was chosen based on AA levels from previously measured urine samples from smokers and nonsmokers. Quantification can only be reported for values that fall within the calibration range (between highest and lowest calibrator levels).

For sample results that are higher than the highest calibrator, the analysis can

be repeated with a lower volume of sample to bring the result within the calibration range.

### C. *Calibration Verification*

The accuracy of the calibration curve is verified by using testing calibrators. The testing calibrators were prepared using native standards purchased from a different vendor. If a different vendor is not available, a different lot of native standards from the same vendor can be used. Three levels of testing calibrators (low, medium, and high) were prepared and tested to verify calibration accuracy. This accuracy test is performed each time a new standard calibration set, or new internal standard stocks or sources are prepared and used for analyte quantitation.

## 8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

### A. *Hamilton- volume verification*

Volume verification is done monthly at: 45 µL, 1000 µL, and 2000 µL. The temperature is measured and used to determine the density of the liquid used. The Ultimate Liquid Class Validation_Venus2.med program and an integrated balance on the Hamilton is used to determine the volume dispensed by the Hamilton. If the %error between the dispensed and set values is less than 5%, then the volume delivered is considered accurate. If the difference is more than 5%, then a service call will be placed with Hamilton. No aliquot can be performed until a service engineer from Hamilton services certifies the volume verification.

### B. *Sample Preparation*

An analytical run consists of 1 water blank, 1 QC low, 1 QC high, and unknown urine samples.

(1) Thaw samples at room temperature if they are frozen.

(2) De-cap samples and load onto Hamilton sample carriers. The following sequence is utilized: water blank, QC low, unknown samples 1-xx, and QC high. This will result in one run/batch with a total of 1 water blank, 1 QC low, 1 QC high, and maximum 29 unknown samples.

(3) Place 1-32 barcoded high recovery vials in one-four sample delivery racks on the Hamilton deck carriers. The barcode is AYYMMDDxx, with xx being 01 to 32.

(4) Place eight 0.5-mL vial of ISTD spiking solution in position 1-8 of

Hamilton carrier.

(5) Scan in the barcodes from the original sample vials and receiving barcoded high recovery vials to generate a Hamilton output file that is used to make a sequence file and run sheet.

(6) Aliquot 2.0 mL of each sample to a corresponding barcode high recovery vial.

(7) Add 45 µL of ISTD and 50 µL of 10 M NaOH to each barcoded vial on the receiving rack.

(8) Cap, vortex, and transfer the 1-32 barcoded vials to the dry heat block, pre-heat at 90ºC. Hydrolyze overnight for approximately 15 hours.

(9) Remove the samples from the heating block. Let cool to room temperature. Vortex samples.

(10) Load the samples back onto the Hamilton carriers as before. The following sequence is utilized: water blank, QC low, unknown samples 1-29, and QC high.

(11) Load each sample to a corresponding SLE cartridge. Wait approximately 10 minutes to let the sample absorb onto the cartridge gravitationally.

(12) Deliver approximately 10 mL DCM to each cartridge, collecting the eluent into 13x100 silanized tube placing directly below each SLE cartridges.

(13) Transfer the siliconized tubes to a Savant, dry down to approximately 250 µL.

(14) Add 3 µL of TMA and 3 µL of PFPA to each sample for derivatization. Cap and vortex, and leave at room temperature for 30 minutes.

(15) Transfer the derivatized sample to a 12x32 mm amber vial with 300 µL insert. Dry down to completion in a Savant.

(16) Reconstitute with 10 µL toluene.

(17) Cap vials with aluminum crimp caps.

(18) Samples are immediately ran on GC-QQQ or store at -20±4°C.

## C. *Sample Analysis*

This assay uses a GC coupled with a triple quadrupole mass spectrometer to quantitate AAs in human urine. The Hamilton output file that was generated during the sample preparation process is converted to a MassHunter sequence file. This sequence file is used to run the prepared batch on an Agilent GC triple quad 7000C.

The analytes are first resolved from other potential interferences on an Agilent J&W DB-17MS column (or comparable column such as Restek, Thermo can be used). Afterwards, further selectivity is accomplished using a triple quadrupole mass spectrometer operated under positive electron impact ionization and multiple reaction monitoring (MRM) mode. Comparison of the area ratio (native analyte area/isotope labeled analyte area) with previously generated calibration curve yields individual analyte concentrations.

Before the run:

(1) Analyze a toluene solvent blank to check for system contamination in the GC/MS-MS system.

(2) A typical run order is as follow: a hexane standard curve, toluene solvent blank, analytical samples, and a toluene solvent blank.

## D. *Processing of Data*

Process all the raw data files using instrument's quantification software (or comparable software package).

(1) Analyzing and Storing the GC/MS-MS Data

a. Upon completion of an analytical run, quantitation will be done via Indigo ASCENT™ quantitation software: selection and integration of quantification and confirmation peaks for all native analytes and the internal standards; sample QC verification such as retention time, internal standard counts, carry over, etc.

b. Review the automated integrations of peaks to ensure correct integration. Manually re-integrate if the integration was chosen incorrectly.

c. Verify and certify the quantitation results.

d. Download Indigo result files to the corresponding run sheet file folder.

e. Upload the Indigo result files to Repeat Manager database per protocol.

f. All Hamilton output files and report data files are stored in the TEB share drive folder: \\cdc\project\CCEHIP_NCEH_DLS_ERATB_TEBL\AA, organized according to study and run sheet name.

(2) Evaluation of Calibration Curves

The y-intercept of each calibration curve should not be significantly different from zero ($p \geq 0.05$); if it is, the source of bias should be identified. An $R^2$ of $\geq 0.98$ is acceptable. Through visual inspection, check to see if any single standard is an outlier. If removal of a point changes the slope or intercept by more than 10% it should be considered an outlier. If either the highest or lowest standard is removed, the reporting limits must be adjusted to reflect the new reporting range.

(3) Evaluation of Quality Control Material

After the completion of a run, the calculated results from the analysis of quality control samples are compared to the established quality control limits to determine if the run is “in control”. Quality control procedures implemented in this method are defined by the Division’s Policies and Procedures Manual (for more information see: Caudill SP, Schleicher RL, and Pirkle JL (2008) Multi-rule quality control for the age-related eye disease study, Stat Med, 27: 4094-4106.). QC samples are subjected to the complete analytical process. The data from these materials are then used to estimate method precision and to assess the magnitude of any time-associated trends. The concentrations of these materials should cover the expected concentration range of the analytes for the method.

g. If both the low and the high QC results are within the 2□ limits, then accept the run.

h. If one of two QC results is outside the 2□ limits, then apply the rules below and reject the run if any condition is met.

i. Extreme Outlier – Run result is beyond the characterization mean +/- 4 $S_i$
ii. 3S Rule - Run result is outside a 3$S_i$ limit
iii. 2S Rule - Both run results are outside the same 2Si limit
iv. 10 X-bar Rule – Current and previous 9 run results are on same side of the characterization mean
v. R 4S Rule – Two consecutive standardized run results differ by more than 4$S_i$. Note: Since runs have a single result per pool for

2 pools, comparison of results for the R 4S rule will be with the previous result within run or the last result of the previous run. Standardized results are used because different pools have different means.

(4) Calculation

Calibration curve of each analyte provides the concentration in pg/total volume. The sample result must be reported as ng/L, therefore, the ng/total volume must be corrected as follow:Final concentration (ng/L) = [(calculated concentration from calibration curve)*(volume of ITSD spiking solution in each sample)*(concentration of ITSD spiking solution)]/[(concentration of ISTD in standard)*(sample volume)]

# 9. REPORTABLE RANGE OF RESULTS

People are exposed to aromatic amines from a variety of sources. A broad range of urinary aromatic amine levels can be expected, extending from <LOD to greater than 10 µg/L. As described below, we designed our method to quantify aromatic amines at the concentrations typically found in smokers and some non-smokers. If an unusually high value is observed that is greater than the highest standard on the curve, that sample is flagged as outside the calibration range and a more dilute sample is re-analyzed if sufficient sample exists (see criteria given in section 10).

## A. *Limit of Detection*

The detection limits for AAs in human samples are determined according to the guideline for determination of limits of detection by the Clinical and Laboratory Standard Institute (CLSI. Protocols for Determination of Limits of Detection and Limits of Quantitation: Approved Guideline. CLSI document EP17-A. Wayne, PA: Clinical and Laboratory Standards Institute, 2004).

## B. *Accuracy*

Neat reference and internal standards are obtained from commercial sources. Stock solution concentrations are based on stated purity using gravimetric analysis.
Accuracy was determined by spiking known amounts of AA standard solution into hexane (accuracy in solution) and urine (accuracy in matrix). The accuracy was calculated by the following formula.

*%bias =100* (observed AA level-expected AA level)/expected AA level*

Criteria for accuracy passing is the same as PT (Section 10c below).

### C. *Precision*

The relative %RSD values calculated over 4-5 runs in 5 days include both within-day and between day error. Acceptable RSD values should consistently fall below 20% for all the analytes. If higher RSD values are obtained, the origin should be investigated and corrective action discussed with supervisor.

### D. *Analytical Specificity*

A high degree of analytical specificity is achieved with this approach. Correct retention times, correct ion mass-to-charge ratios, and correct precursor/product ion transitions help ensure a very high degree of specificity and minimize the influence from any potential interference.

An established range of ratios of the response of quantitation ion to that of confirmation ion of QC samples is used to determine if an unknown sample test positive for a given analyte.

### E. *Recovery*

Sample matrix effects for each analyte are evaluated. Spiking same amounts of isotope labeled AA internal standards in urine samples. Prepare these spiked samples according to sample preparation procedures. % recovery is calculated as the ratio of the responses of labeled internal standards (area count) in the urine samples to the responses of internal standard in the standards. The average recovery for all AAs is ranging from 30% to 50%.

### F. *Linearity Limits*

The AA calibration curves established are linear over the concentration ranges from the low and high standard with R2 values greater than or equal to 0.98. The lower reportable limit is either the LoD or the lowest standard concentration, whichever is higher. The upper reportable limit is the highest standard concentration. A residual plot of the calibrators is checked to confirm linearity.

### G. *Ruggedness test*

Ruggedness testing was performed to assess the potential of important analytical variables to affect results. Each of these variables was systematically varied to examine their influence, if any, on the analytical results and was optimized to achieve sensitivity and high throughput.

## 10. QUALITY ASSESSMENT AND PROFICIENCY TESTING

## A. *Quality Assessment*

Quality assessment procedures follow standard practices.

Examine the blank to check for possible contamination in the system or extraction solution or reagents. Next, evaluate standards obtained under the same instrument conditions as that for samples to check the performance of the GC/MS-MS system. If the retention times and peak intensities of the analytes are within the acceptable ranges described in section 8.b., then evaluate sample blanks, QCs and unknown samples in the run sequence.

Compare the QC results obtained from the run with the acceptance criteria to assure the proper operation of the analysis. If a QC result is “out of control”, the cause of the failure should be determined. No results from the associated batch may be reported.

## B. *Establishing QC Limits*

As per division policy, acceptable QC concentration limits must be calculated from the concentration results observed in at least 20 characterization runs. During the 20 characterization runs, previously characterized QCs or pools with target values assigned by outside laboratories should be included to evaluate the analysis. The process of limits calculation is performed using the laboratory database and the SAS division QC characterization program (for more information see: Caudill SP, Schleicher RL, and Pirkle JL (2008) Multi-rule quality control for the age-related eye disease study, Stat Med, 27: 4094-4106.).

## C. *Proficiency Testing*

(1) PT is conducted twice a year. At this time there are no external PT programs or certified reference materials for aromatic amines in human urine. Therefore, we developed an in-house PT program administered by a quality control officer. PT samples are spiked urine samples at three different levels cover the calibration range (more details in Appendix A).

(2) When a PT test is conducted, five PT samples are blind-coded by a QC officer. The five blind-coded PT samples are prepared as unknown samples by an analyst or a team as described in Section 8a. A correct determination, within ±20% of the known concentration, on at least 80% of the samples must be achieved to be considered proficient.

(3) Performance in the PT program along with documentation of remedial action taken for unacceptable performance is to be documented in a QC

Manual in the laboratory that is available for review.

# 11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

If the calibration or QC fails, all operations are suspended until the source or cause of failure is identified and corrected. Analytical results are not reported. After calibration and/or quality control have been reestablished, analytical runs may be resumed.

## A. *Internal Standard Response*

If the area counts of the internal standards of the check standard fall below the established optimal absolute counts, or if the chromatograph peak start to have tailing, this indicates that the instrumental sensitivity has fallen below acceptable limits. The following steps should be taken, and the instrument sensitivity is checked after each step is performed. Once sensitivity has been reestablished, further steps are not necessary.

(1) Replace injection liner & o-ring, and septum

(2) Clean the injection port and needle guide

(3) Trim the GC column

(4) Replace the syringe wash vials and rinse or replace the syringe

(5) Bake out the ion source and quad

(6) Bake out the MMI

(7) Tune the mass spectrometer

(8) Clean the mass spectrometer ion source

(9) Clean the mass spectrometer quads (this must be done by a service engineer)

(10) If the sensitivity is lowered due to band broadening, inspect all

(11) GC connections, leak test, and consider changing the analytical column.

### B. *Calibration Regression*

If the linearity of the calibration curve criterion 0.98 is not met, check if the standards are prepared correctly or if an instrument malfunction has occurred. If no error is found in standard preparation, check if the detector is saturated. Also check if GC delivery pressure is deviated-. Other instrument specific factors that could cause calibrations problems, such as leak, should be checked and corrective action is taken as needed.

### C. *Analyte in Standards or QC Materials*

If an unexpectedly large amount of analyte is measured in one of the calibration standards or QC materials, but is not seen in the remainder of the samples, this indicates a contamination of this particular sample. The source of this contamination should be investigated to prevent repeat occurrences, but no further action is required.

### D. *Analyte in All Samples*

If an unexpectedly large amount of analyte is present in all measurements for a particular day, it is likely that one or more of the solvents or reagents used are contaminated. If necessary, prepare new solvents and/or reagents.

### E. *QC Sample Outside of Control Limits*

Verify the integrity of the QC material if the result of QC sample falls outside the control limits. Check if the proper amount of internal standard was added to that sample. Also confirm that the integration was performed correctly. No analytical results can be reported for runs that QC is outside of the control limits.

## 11. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

Some plastic labware, solvents, air and water may contain trace amounts of AAs which could contaminate urine samples. We guard against biased data by screening reagents and laboratory materials that come in contact with samples. In addition, the specificity of detection by GC/EI-MS/MS helps to avoid background chemical interferences. It is highly unlikely that another substance would have the same mass transitions, retention times, and relative abundances of different MRMs as any of our analytes.

## 12. REFERENCE RANGES (NORMAL VALUES)

The study population typically includes both smokers and non-smokers, therefore, a large range of urinary aromatic amine levels are expected. Current literature reported mean levels for nonsmokers and smokers are listed in the

table below. (9-10, 12). We plan to apply the method described in this document to characterize population-based ranges for U.S. smokers and non-smokers.

| Analyte | Nonsmoker (ng/L) | Smoker (ng/L) |
|---|---|---|
| ***o*-TOL** | 55 | 117 |
| **2-AMN** | 5 | 12 |
| **4-ABP** | 2 | 9 |

## 13. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable for this procedure.

## 14. SPECIMEN STORAGE AND HANDLING DURING TESTING

Samples are received refrigerated or frozen and stored frozen at -70±10°C. If the entire sample volume is not used in the assay, then residual urine is refrozen and stored at -70±10°C. All analytes were tested for stability for at least 5 thawing-re-freezing cycles as specified in section h in the Appendix B.

## 15. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

If a problem with the method exists, samples are held in the freezer until it can be resolved. If necessary, extracted and derivatized samples ready for analysis can be sealed and stored at -20ºC±4°C up to one year before they are analyzed.

## 16. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable.

## 17. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

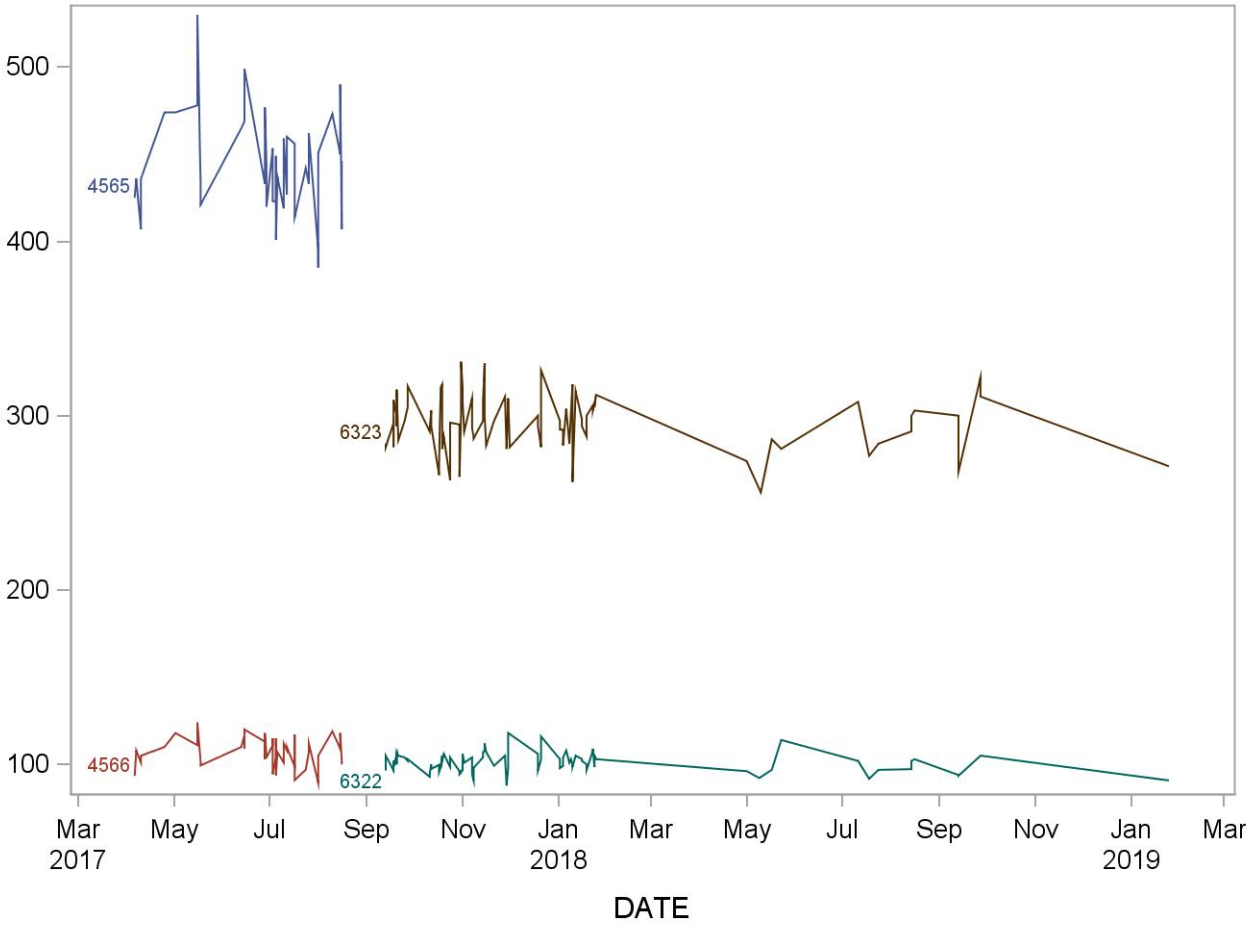
Any residual urine is stored at -70±10°C.

## 18. SUMMARY STATISTICS AND QC GRAPHS

See following pages.

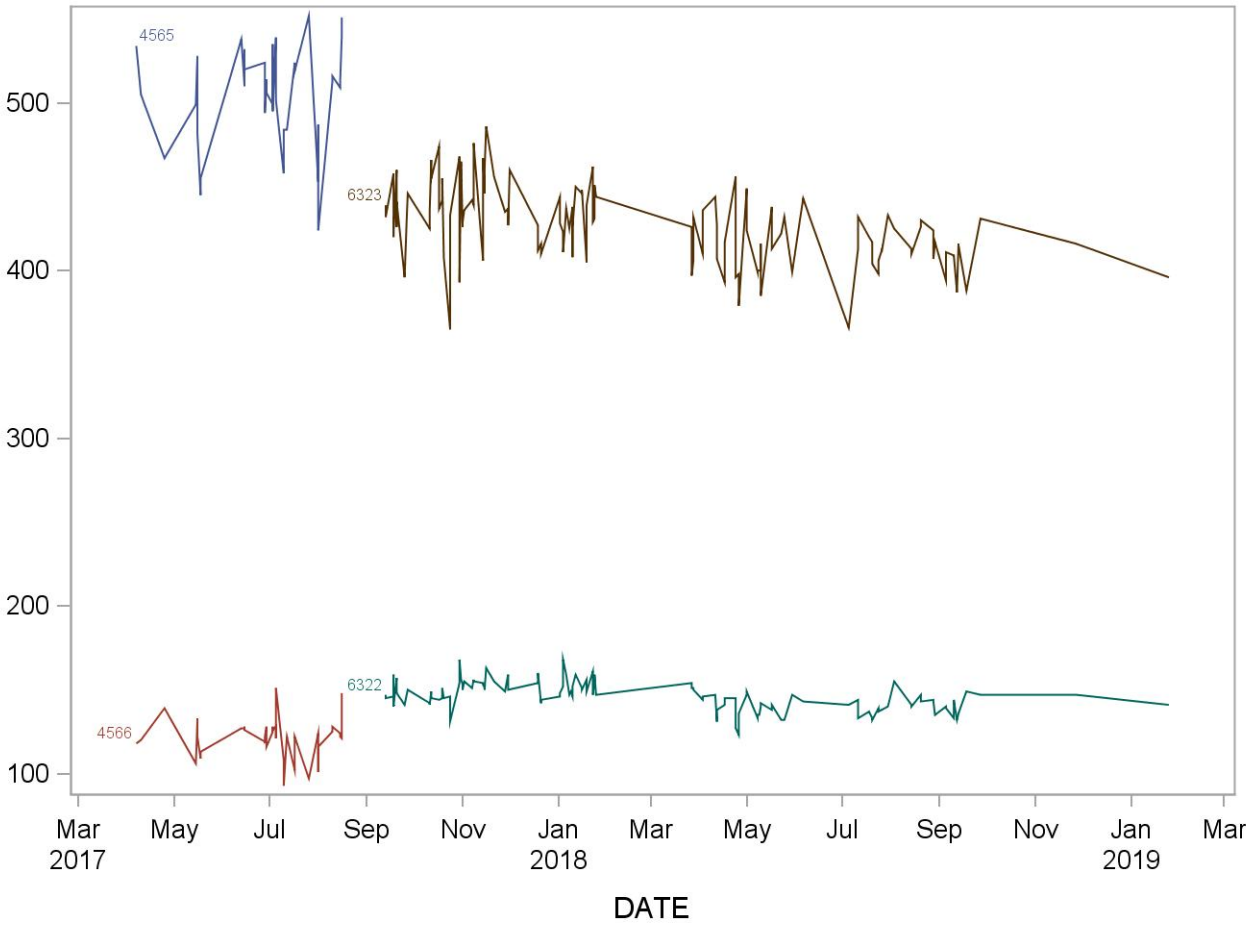
## 2013-2014 Summary Statistics and QC Chart for 1-Aminonaphthalene, urine (pg/mL)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| 4565 | 46 | 06APR17 | 16AUG17 | 445.000 | 28.758 | 6.5 |
| 4566 | 46 | 06APR17 | 16AUG17 | 106.591 | 8.452 | 7.9 |
| 6323 | 85 | 13SEP17 | 25JAN19 | 295.424 | 15.974 | 5.4 |
| 6322 | 85 | 13SEP17 | 25JAN19 | 100.893 | 5.706 | 5.7 |



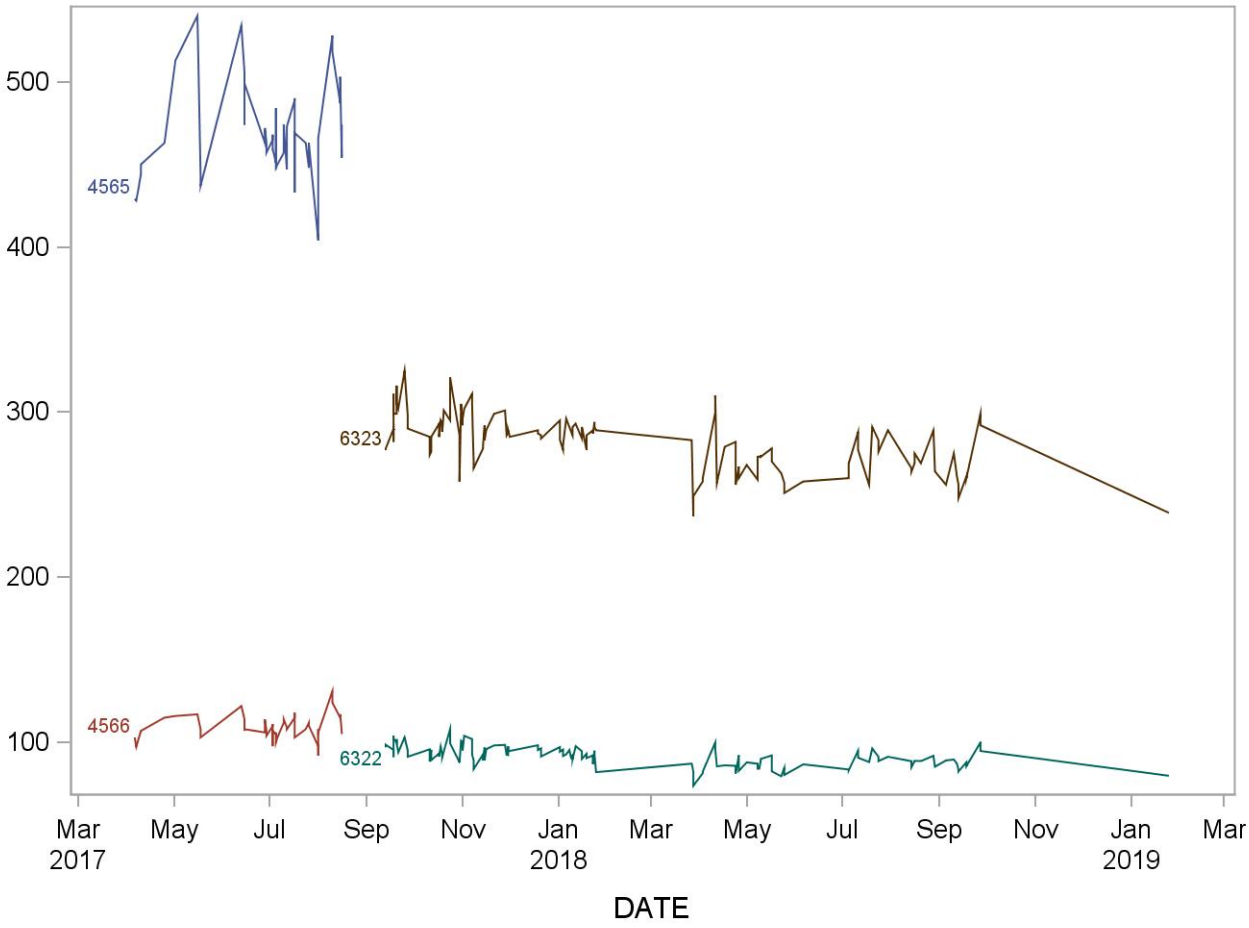
## 2013-2014 Summary Statistics and QC Chart for 2,6-Dimethylaniline, urine (pg/mL)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| 4565 | 40 | 07APR17 | 16AUG17 | 503.775 | 29.528 | 5.9 |
| 4566 | 40 | 07APR17 | 16AUG17 | 121.273 | 11.706 | 9.7 |
| 6323 | 123 | 13SEP17 | 25JAN19 | 425.374 | 23.127 | 5.4 |
| 6322 | 123 | 13SEP17 | 25JAN19 | 145.772 | 8.411 | 5.8 |



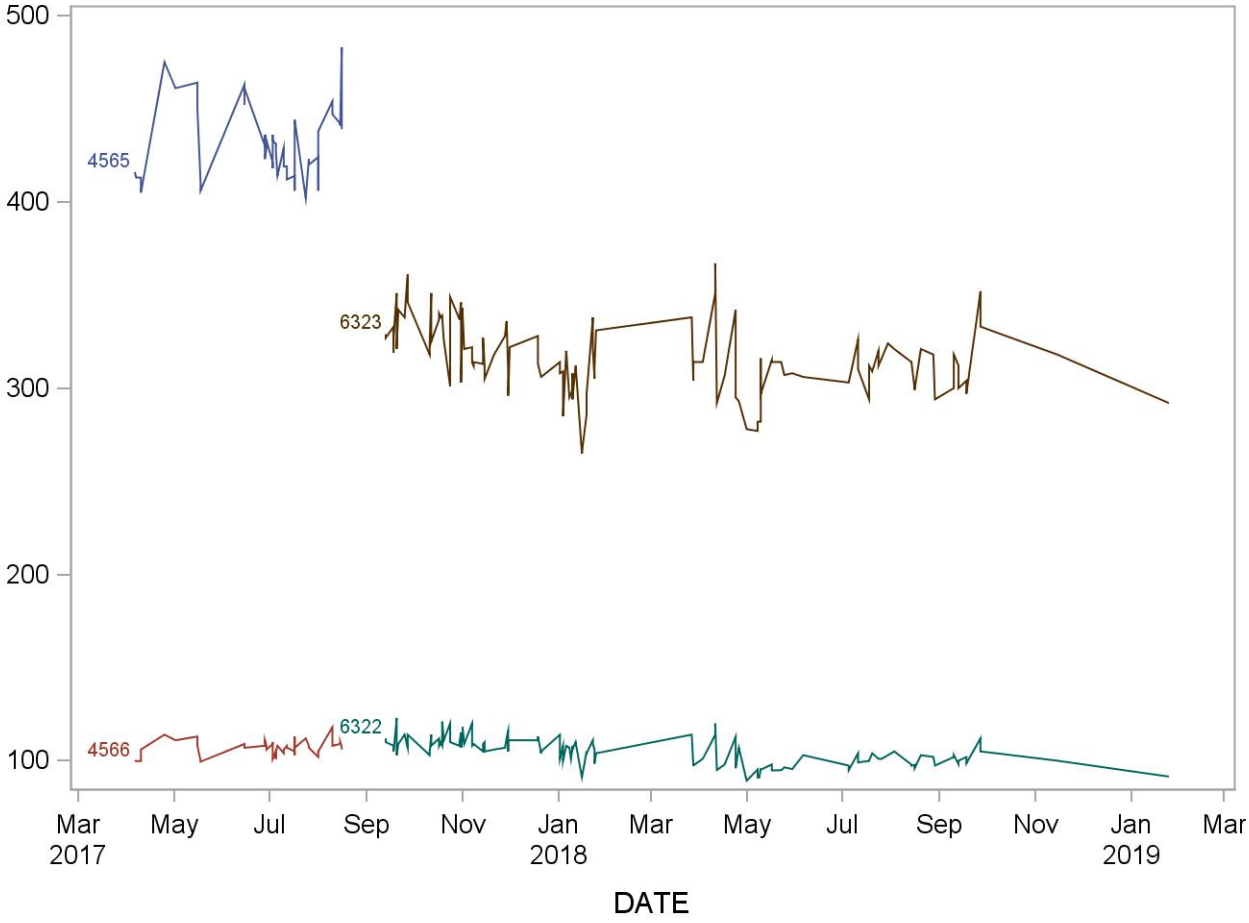
## 2013-2014 Summary Statistics and QC Chart for 2-Aminonaphthalene, urine (pg/mL)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| 4565 | 47 | 06APR17 | 16AUG17 | 467.596 | 29.027 | 6.2 |
| 4566 | 47 | 06APR17 | 16AUG17 | 108.972 | 7.177 | 6.6 |
| 6323 | 122 | 13SEP17 | 25JAN19 | 281.139 | 17.297 | 6.2 |
| 6322 | 122 | 13SEP17 | 25JAN19 | 91.437 | 6.281 | 6.9 |



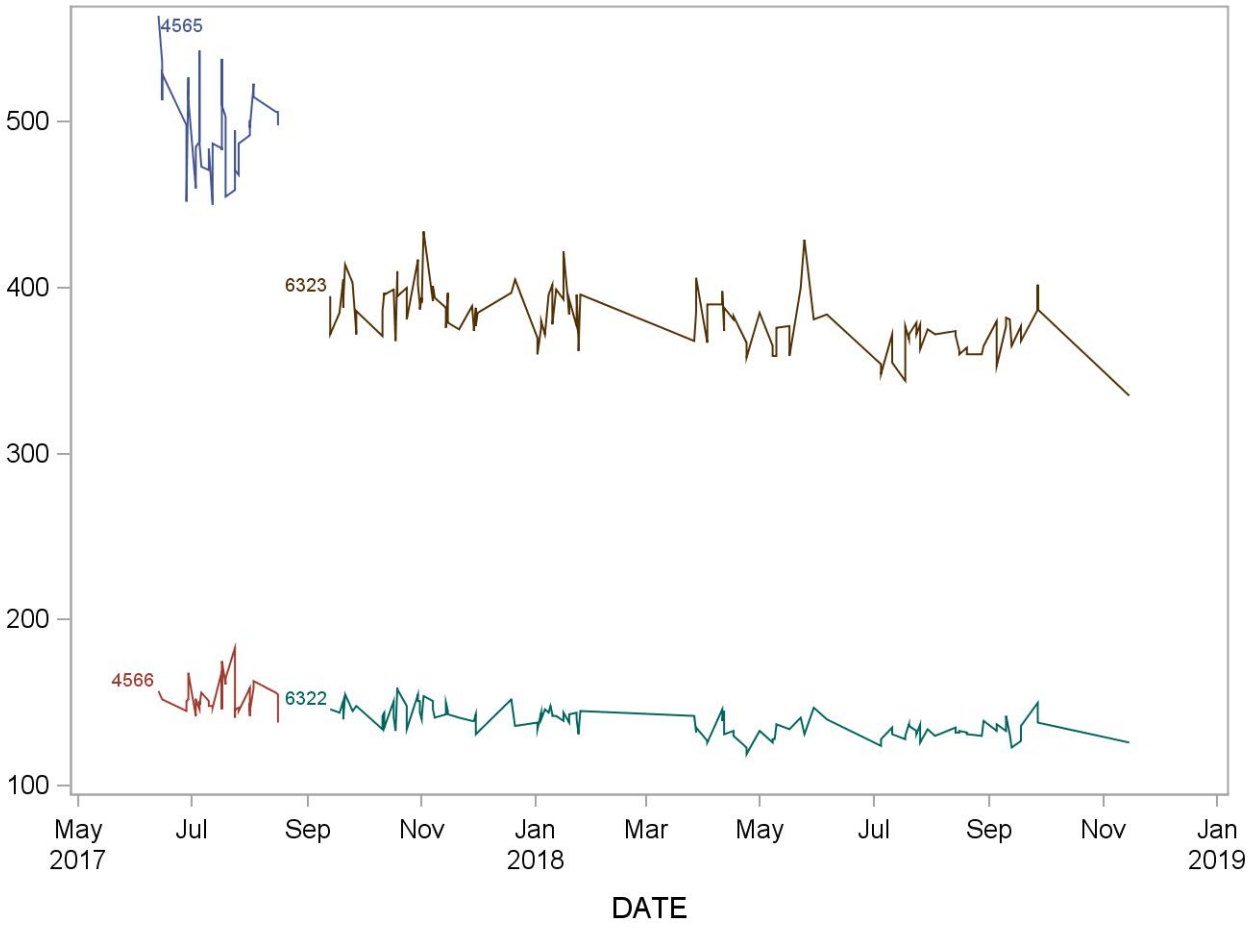
## 2013-2014 Summary Statistics and QC Chart for 4-Aminobiphenyl, urine (pg/mL)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| 4565 | 47 | 06APR17 | 16AUG17 | 431.340 | 19.378 | 4.5 |
| 4566 | 47 | 06APR17 | 16AUG17 | 106.747 | 4.019 | 3.8 |
| 6323 | 122 | 13SEP17 | 25JAN19 | 316.557 | 18.826 | 5.9 |
| 6322 | 122 | 13SEP17 | 25JAN19 | 104.941 | 7.159 | 6.8 |



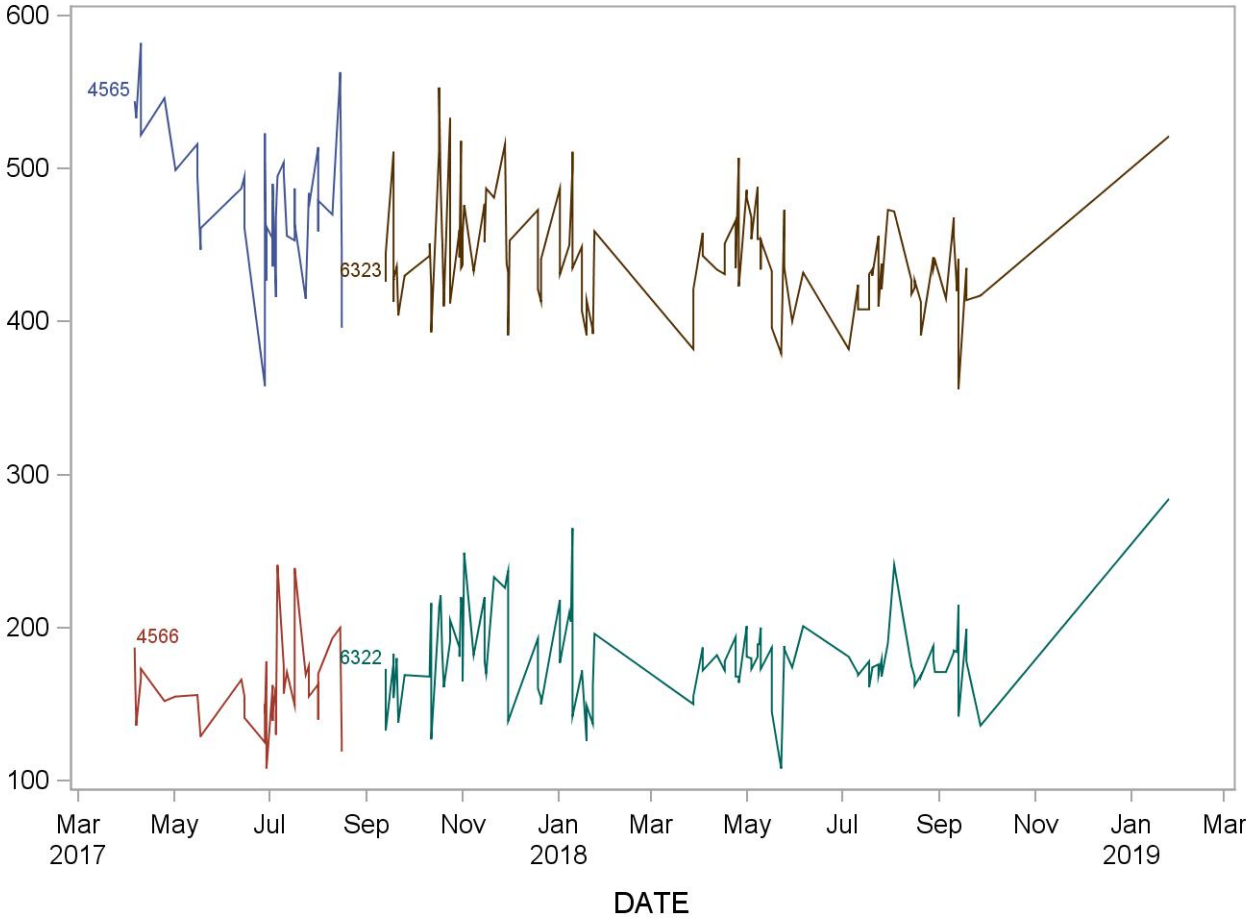
## 2013-2014 Summary Statistics and QC Chart for o-Anisidine, urine (pg/mL)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| 4565 | 41 | 13JUN17 | 16AUG17 | 494.659 | 26.163 | 5.3 |
| 4566 | 41 | 13JUN17 | 16AUG17 | 153.146 | 9.843 | 6.4 |
| 6323 | 122 | 13SEP17 | 15NOV18 | 381.885 | 17.088 | 4.5 |
| 6322 | 122 | 13SEP17 | 15NOV18 | 138.648 | 8.180 | 5.9 |



## 2013-2014 Summary Statistics and QC Chart for o-Toluidine, urine (pg/mL)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| 4565 | 43 | 06APR17 | 16AUG17 | 479.186 | 43.314 | 9.0 |
| 4566 | 43 | 06APR17 | 16AUG17 | 158.953 | 26.781 | 16.8 |
| 6323 | 114 | 13SEP17 | 25JAN19 | 443.167 | 36.197 | 8.2 |
| 6322 | 114 | 13SEP17 | 25JAN19 | 182.211 | 29.761 | 16.3 |



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# Appendix A

## Standard Materials

### *A. Stock reagent preparation*

Each analyte was ordered separately in pure form (vendors and purity shown below).

(1) Native standard

Stock solutions of individual analyte and subsequent combined analyte dilutions are prepared in ethanol. The following table shows an example how the stock solutions (A) and combined analyte dilutions (B, C, & D) are made. B contained only OTOL to make standards with higher levels of OTOL.

Table A1:

| Date 1mg/mL Stock Made | Analyte | Vendor | Chemical Purity (%) | Purity Test | Lot | Analyte Weight | Stock A Conc. | Vol. of Stock A in Dilution B | Conc. of Analyte in Dilution B | Vol. of Stock A in Dilution C | Conc. of Analyte in Dilution C | Vol. of Stock A in Dilution D | Conc. of Analyte in Dilution D |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | (mg) | (mg/mL) | (µL) | (ng/µL) | (µL) | (ng/µL) | (µL) | (ng/µL) |
| 03/31/15 | OTOL | Fluka | 99.5 | GC | 1421704V | 10.720 | 1.0666 | 500.000 | 106.664 | 235.000 | 10.026 | 95.000 | 1.013 |
| 07/28/14 | 26DM | Aldrich | 98.5 | GC | STBB5452V | 7.840 | 0.7722 | | | 325.000 | 10.039 | 130.000 | 1.004 |
| 07/28/14 | OANS | Fluka | 99.7 | GC | SZBB343XV | 9.630 | 0.9601 | | | 260.000 | 9.985 | 105.000 | 1.008 |
| 07/28/14 | QNL | Aldrich | 97.5 | GC | MKBL8695V | 9.330 | 0.9097 | | | 275.000 | 10.006 | 110.000 | 1.001 |
| 03/31/15 | 1AMN | Fluka | 99.9 | GC | SZB8268XV | 10.100 | 1.0090 | | | 245.000 | 9.888 | 100.000 | 1.009 |
| 03/31/15 | 2AMN | Sigma | 98.0 | TLC | SLBD0851V | 11.090 | 1.0868 | | | 230.000 | 9.999 | 90.000 | 0.978 |
| 07/28/14 | 4ABP | Aldrich | 99.0 | TLC | 128K1386V | 10.830 | 1.0722 | | | 230.000 | 9.864 | 90.000 | 0.965 |

*Final volume of stock A was 10 mL in ethanol*
*Final volume of stock B was 5 mL in ethanol*
*Final volume of Dilution C was 25 mL in ethanol*
*Final volume of Dilution D was 100 mL in ethanol*

Table A2:

Prepare one complete set of calibration standards in hexane (B&J, lot DH930) as described in detail below. A total of 18 standards were prepared ranging from 0 to 1,300 pg/µL.

The concentration of AA in standard 1-17 is listed in table A3. Standard 14-17 contain only OTOL to accommodate higher levels of OTOL that were observed in unknown samples.

Table A3:

| Analyte | Std. No. 1 | Std. No. 2 | Std. No. 3 | Std. No. 4 | Std. No. 5 | Std. No. 6 | Std. No. 7 | Std. No. 8 | Std. No. 9 | Std. No. 10 | Std. No. 11 | Std. No. 12 | Std. No. 13 | Std. No. 14 | Std. No. 15 | Std. No. 16 | Std. No. 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (pg/µL) | (pg/µL) | (pg/µL) | (pg/µL) | (pg/µL) | (pg/µL) | (pg/µL) | (pg/µL) | (pg/µL) | (pg/µL) | (pg/µL) | (pg/µL) | (pg/µL) | (pg/µL) | (pg/µL) | (pg/µL) | (pg/µL) |
| OTOL | 0.5 | 2.5 | 5.1 | 7.1 | 10.1 | 20.1 | 25.1 | 30.1 | 40.1 | 50.1 | 75.2 | 100.3 | 213.3 | 426.6 | 853.3 | 1,066.60 | 1,279.90 |
| 26DM | 0.5 | 2.5 | 5.0 | 7.0 | 10.0 | 20.1 | 25.1 | 30.1 | 40.2 | 50.2 | 75.3 | 100.4 | 200.8 | | | | |
| OANS | 0.5 | 2.5 | 5.0 | 7.1 | 10.1 | 20.0 | 25.0 | 30.0 | 39.9 | 49.9 | 74.9 | 99.9 | 199.7 | | | | |
| 1AMN | 0.5 | 2.5 | 5.0 | 7.1 | 10.1 | 19.8 | 24.7 | 29.7 | 39.6 | 49.4 | 74.2 | 98.9 | 197.8 | | | | |
| 2AMN | 0.5 | 2.4 | 4.9 | 6.8 | 9.8 | 20.0 | 25.0 | 30.0 | 40.0 | 50.0 | 75.0 | 100.0 | 200.0 | | | | |
| 4ABP | 0.5 | 2.4 | 4.8 | 6.8 | 9.6 | 19.7 | 24.7 | 29.6 | 39.5 | 49.3 | 74.0 | 98.6 | 197.3 | | | | |

*All standards were made at a volume of 50 mL, except standard 1 and 2 which were made at a volume of 100 mL.*

| Standard Number | Standard Conc. (pg/µL) | Final Standard Volume (mL) | Dilution Used | Vol. of Dilution Added (µL) |
|---|---|---|---|---|
| 0 | 0.000 | 0.000 | NA | 50.000 |
| 1 | 0.500 | 100.000 | D | 50.000 |
| 2 | 2.500 | 100.000 | D | 250.000 |
| 3 | 5.000 | 50.000 | D | 250.000 |
| 4 | 7.000 | 50.000 | D | 350.000 |
| 5 | 10.000 | 50.000 | D | 500.000 |
| 6 | 20.000 | 50.000 | C | 100.000 |
| 7 | 25.000 | 50.000 | C | 125.000 |
| 8 | 30.000 | 50.000 | C | 150.000 |
| 9 | 40.000 | 50.000 | C | 200.000 |
| 10 | 50.000 | 50.000 | C | 250.000 |
| 11 | 75.000 | 50.000 | C | 375.000 |
| 12 | 100.000 | 50.000 | C | 500.000 |
| 13 | 200.000 | 50.000 | C | 1000.000 |
| 13 | 213.328 | 50.000 | B | 100.000 |
| 14 | 426.656 | 50.000 | B | 200.000 |
| 15 | 853.312 | 50.000 | B | 400.000 |
| 16 | 1066.640 | 50.000 | B | 500.000 |
| 17 | 1279.968 | 50.000 | B | 600.000 |

1) Accuracy testing solutions

To test the accuracy of our calibration curve, accuracy testing solutions were prepared using native stocks that were purchased from different vendors. If a different vendor was not commercially available, then a different lot from the same vendor was purchased and used.

Table A4: Accuracy testing stocks and solutions

| Date stock made | Analyte | Vendor | Lot | Purity (%) | Actual Weight | Stock A conc. (mg/mL) | Volume stock A used in C2 (µL) | Dilution C2 conc. (ng/µL) | Volume stock A used in D2 (µL) | Dilution D2 conc. (ng/µL) |
|---|---|---|---|---|---|---|---|---|---|---|
| 7/28/2014 | oTOL | TRC | 14-ANR-105 | 97.0 | 11.540 | 1.1194 | 220.000 | 9.851 | 90.000 | 1.007 |
| 7/28/2014 | 26DM | TRC | 12-ABY-5-1 | 98.0 | 8.410 | 0.8242 | 245.000 | 8.077 | 120.000 | 0.989 |
| 7/28/2014 | oANS | TCI | SDGQC | 99.7 | 9.400 | 0.9372 | 265.000 | 9.934 | 105.000 | 0.984 |
| 7/28/2014 | 1AMN | Fluka | 7080X | 99.9 | 10.180 | 1.0170 | 210.000 | 8.543 | 100.000 | 1.017 |
| 7/28/2014 | 2AMN | TRC | 6-GNM-1-2 | 95.0 | 14.620 | 1.3889 | 180.000 | 10.000 | 70.000 | 0.972 |
| 7/28/2014 | 4ABP | Sigma | 056K1361 | 99.0 | 10.630 | 1.0524 | 240.000 | 10.103 | 95.000 | 1.000 |

*Stock A was made in 10 mL ethanol.*
*Dilution C2 was made in 25 mL ethanol.*
*Dilution D2 was made in 100 mL ethanol.*

| Testing ID | Conc. (pg/uL) | Final Volume (mL) | Stock Used | Stock Volume (µL) |
|---|---|---|---|---|
| ACC-1 | 2.500 | 100.000 | D | 250.000 |
| ACC-2 | 7.000 | 50.000 | D | 350.000 |
| ACC-3 | 25.000 | 25.000 | D | 650.000 |
| ACC-4 | 50.000 | 50.000 | C | 250.000 |
| ACC-5 | 200.000 | 25.000 | C | 500.000 |

Table A5: Accuracy testing solution concentrations

| Analyte | ACC-1 | ACC-2 | ACC-3 | ACC-4 | ACC-5 |
|---|---|---|---|---|---|
| oTOL | 2.519 | 7.052 | 26.193 | 49.253 | 197.011 |
| 26DM | 2.510 | 7.027 | 25.714 | 40.385 | 161.539 |
| oANS | 2.520 | 7.057 | 25.585 | 49.671 | 198.682 |
| 1AMN | 2.503 | 7.008 | 26.442 | 42.713 | 170.853 |
| 2AMN | 2.423 | 6.783 | 25.278 | 50.000 | 200.002 |
| 4ABP | 2.412 | 6.755 | 25.994 | 50.514 | 202.055 |

2) Isotopically Labeled Internal Standards
*All concentrations are calculated with correction for percentage of chemical and isotopic purity.*

Table A6:

| Date 1mg/mL Made | Analyte | Vendor | Chemical Purity (%) | Isotopic Purity (%) | Purity Test | Lot | Actual Weight (mg) | Stock DA Final Con.* 10 ml (mg/mL) |
|---|---|---|---|---|---|---|---|---|
| 01/29/15 | OTOL-$^{13}C_6$ | MI | 98.0 | 98.0 | Mass Spec | 713 | 2.360 | 0.2267 |
| 07/28/14 | PTOL-$D_9$ | MI | 98.6 | 99.0 | GC | 18 | 9.230 | 0.9010 |
| 07/28/14 | MTOL-$D_9$ | MI | 98.4 | 99.0 | GC | 310 | 9.070 | 0.8836 |
| 07/28/14 | 26DM-$D_6$ | TRC | 98.0 | 95.3 | Mass Spec | 3-MIC-149-1 | 9.000 | 0.8405 |
| 07/28/14 | OANS-$D_7$ | MI | 99.8 | 99.4 | HPLC & NMR | 533 | 10.180 | 1.0099 |
| 01/29/15 | 2ABP-$D_9$ | MI | 99.0 | 99.6 | HPLC | 932 | 10.430 | 1.0284 |
| 07/28/14 | 1AMN-$D_9$ | MI | 98.1 | 99.1 | HPLC & NMR | 145 | 10.790 | 1.0490 |
| 12/21/15 | 2AMN-$D_7$ | CDN | 98.0 | 95.0 | NMR & Mass Spec | Z-330 | 3.170 | 0.3170 |
| 07/28/14 | 3ABP-$D_9$ | TRC | 98.0 | 98.7 | Mass Spec | 12-SDJ-187-1 | 8.700 | 0.8415 |
| 07/28/14 | 4ABP-$D_9$ | CI | 99.0 | 99.6 | HPLC & NMR | I1-9733 | 9.570 | 0.9436 |

a. Internal standard spiking solution for standard solutions

Table A7:

| Date made | Analyte | Stock A Final Con. (mg/mL) | Volume Stock A used (µL) | [1]Final DC Conc. (mg/mL) | [1]Final DC Conc. (ng/µL) | [2]Final DD conc (ng/µL) | [3]Final conc of ISTD in ea STD solution (ng/mL or pg/µL) |
|---|---|---|---|---|---|---|---|
| 12/21/2015 | OTOL-$^{13}C_6$ | 0.227 | 1100.000 | 0.010 | 9.973 | 0.997 | 39.891 |
| 12/21/2015 | PTOL-$D_9$ | 0.901 | 300.000 | 0.011 | 10.812 | 1.081 | 43.247 |
| 12/21/2015 | MTOL-$D_9$ | 0.884 | 300.000 | 0.011 | 10.603 | 1.060 | 42.411 |
| 12/21/2015 | 26DM-$D_6$ | 0.841 | 300.000 | 0.010 | 10.087 | 1.009 | 40.346 |
| 12/21/2015 | OANS-$D_7$ | 1.010 | 300.000 | 0.012 | 12.118 | 1.212 | 48.474 |
| 12/21/2015 | 2ABP-$D_9$ | 1.028 | 300.000 | 0.012 | 12.341 | 1.234 | 49.365 |
| 12/21/2015 | 1AMN-$D_9$ | 1.049 | 300.000 | 0.013 | 12.588 | 1.259 | 50.351 |
| 12/21/2015 | 2AMN-$D_7$ | 0.317 | 1000.000 | 0.013 | 12.680 | 1.268 | 50.720 |
| 12/21/2015 | 3ABP-$D_9$ | 0.842 | 250.000 | 0.008 | 8.415 | 0.842 | 33.661 |
| 12/21/2015 | 4ABP-$D_9$ | 0.944 | 300.000 | 0.011 | 11.324 | 1.132 | 45.295 |

*[1]DC was made in 25 mL ethanol*
*[2]DD was made by 1:10 dilution of DC in hexane*
*[3]200 µl of DD was added to each 5 mL standard solution*
*Toluene. Burdick & Jackson Microsolve VLSI MS80863-4 GC 99.9% purity.*
*Hexane. Burdick & Jackson 216-4 High Purity*

### *a. Derivatization of calibrators and accuracy testing solutions*

Aliquot 5mL of each calibration standard, 0-13, and accuracy testing solutions,

ACC-1 to ACC-5. An aliquot of hexane solvent blank was also added.
Add 200µL of ITSD DD solution in each aliquot.
Add 25 µL of TMA and 25 µL of PFPA, wait 30 minutes.
Transfer to siliconized tubes, dry completely in a Savant, reconstituted to 5 ml toluene using volumetric flasks: rinsing each siliconized tube 2x2 ml with toluene and bring up to final 5 ml.
Aliquot to 5 sets of 1 mL in high recovery amber vial.

### *b. Internal standard spiking solution for unknown samples:*

Internal standard (ISTD) spiking solution for unknown sample preparation was prepared from stock DC. Spiking solution was dispensed in 4.0 ml aliquots and stored frozen at -20±4°C. The exact concentration of each isotopically labeled analyte in the ITSD spiking solution is shown below.

Table A8:

| Date made | Analyte | Final DC Conc. (ng/µL) | Volume of Stock DC used (µL) | Spiking solution conc. (ng/mL) or (pg/µL) | Total amount ISTD added in ea sa (pg) |
|---|---|---|---|---|---|
| 12/21/2015 | OTOL-$^{13}C_6$ | 9.973 | 1000.000 | 9.973 | 448.776 |
| 12/21/2015 | PTOL-$D_9$ | 10.812 | 1000.000 | 10.812 | 486.528 |
| 12/21/2015 | MTOL-$D_9$ | 10.603 | 1000.000 | 10.603 | 477.124 |
| 12/21/2015 | 26DM-$D_6$ | 10.087 | 1000.000 | 10.087 | 453.895 |
| 12/21/2015 | OANS-$D_7$ | 12.118 | 1000.000 | 12.118 | 545.329 |
| 12/21/2015 | 2ABP-$D_9$ | 12.341 | 1000.000 | 12.341 | 555.357 |
| 12/21/2015 | 1AMN-$D_9$ | 12.588 | 1000.000 | 12.588 | 566.445 |
| 12/21/2015 | 2AMN-$D_7$ | 12.680 | 1000.000 | 12.680 | 570.600 |
| 12/21/2015 | 3ABP-$D_9$ | 8.415 | 1000.000 | 8.415 | 378.682 |
| 12/21/2015 | 4ABP-$D_9$ | 11.324 | 1000.000 | 11.324 | 509.566 |

*Dilution DC was made in 25 mL ethanol.*
*Final spiking solution was made in 1L ethanol.*
*45 µl is spiked in each unknown sample.*

## *B. Controls*

### *1. Quality control materials*

There are two quality control pools for the urinary aromatic amines assay. Pools QC Low and “QC High contain relatively low and high levels of urinary aromatic amines, respectively. The QC pools were prepared in-house from urine collected from non-smokers, filtered with 0.2µm filters and spiked with combined analyte standard stock solution. Each pool was mixed well, dispensed in 2.4mL aliquots into 5mL cryovials, and stored frozen at -

70±10°C.

Table B1: Concentration of AA in QC pools

| Pool ID | Stock Dilution used for Spiking | Amount Spiked (µL) | Final Volume of Urine (mL) | Final Pool Desired Conc. (pg/mL) |
|---|---|---|---|---|
| QC_Low2015 | D* | 150 | 1200 | 125 |
| QCHigh_2015 | C* | 60 | 1200 | 500 |
| QCLow_2016 | D* | 350 | 3,200 | 100 |
| QCHigh_2016 | C* | 125 | 3,200 | 400 |

**Dilution D and C are the same Dilution stocks used to make standard solution (Table A1)*

| Date 1mg/mL Stock Made | Analyte | Vendor | Lot | Weight (mg) | Stock A Conc. (mg/mL) | Vol Sotck A used in Dil C (µL) | Dilution C Conc. (ng/µL) | Vol Sotck A used in Dil D (µL) | Dilution D Conc. (ng/µL) | QC_Low2015 (pg/mL) | QCHigh_2015 (pg/mL) | QCLow_2016 (pg/mL) | QCHigh_2016 (pg/mL) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 08/31/15 | OTOL | Fluka | 1421704V | 10.720 | 1.0666 | 235.000 | 10.026 | 95.000 | 1.013 | 126.664 | 501.321 | 110.831 | 391.657 |
| 07/28/14 | 26DM | Aldrich | STBB5452V | 7.840 | 0.7722 | 325.000 | 10.039 | 130.000 | 1.004 | 125.489 | 501.956 | 109.803 | 392.153 |
| 07/28/14 | OANS | Fluka | SZBB343XV | 9.630 | 0.9601 | 260.000 | 9.985 | 105.000 | 1.008 | 126.015 | 499.258 | 110.263 | 390.045 |
| 08/31/15 | 1AMN | Fluka | SZB8268XV | 10.100 | 1.0090 | 245.000 | 9.888 | 100.000 | 1.009 | 126.124 | 494.405 | 110.358 | 386.254 |
| 08/31/15 | 2AMN | Sigma | SLBD0851V | 11.090 | 1.0868 | 230.000 | 9.999 | 90.000 | 0.978 | 122.267 | 499.937 | 106.984 | 390.576 |
| 07/28/14 | 4ABP | Aldrich | 128K1386V | 10.830 | 1.0722 | 230.000 | 9.864 | 90.000 | 0.965 | 120.619 | 493.198 | 105.542 | 385.311 |

*Stock A was made in 10 mL ethanol.*
*Dilution C was made in 25 mL ethanol.*
*Dilution D was made in 100 mL ethanol.*

### *2. Limit of Detection Materials*

There are three limit of detection pools for the urinary aromatic amines assay. Pools “LoD1” and “LoD2” and “LoD3”contain relatively low, medium and high levels of urinary aromatic amines, respectively. The LoD pools were prepared in-house from urine collected from non-smokers, filtered with 0.2µm filters and spiked with standard stock solution. Each pool was mixed well, dispensed in 2.4mL aliquots into 5mL cryovials with screw cap, and stored frozen at -60°C to -70°C.

Table B2: Concentration of LoD pools

| Pool ID | Stock Dilution used for Spiking | Amount Spiked (µL) | Final Volume of Urine (mL) | Final Pool Desired Conc. (pg/mL) |
|---|---|---|---|---|
| LoD0 | | 0 | 300 | TBD, background matrix |
| LoD1 | E | 60 | 300 | 20 |
| LoD2 | E | 120 | 300 | 40 |
| LoD3 | E | 180 | 300 | 60 |

| Date 1mg/mL Stock Made | Analyte | Vendor | Lot | Weight (mg) | Stock A Conc. (mg/mL) | Vol Sotck A used in Dil D (µL) | Dilution D Conc. (ng/µL) | Dilution E conc. (pg/µL) | LOD 1 (pg/mL) | LOD 2 (pg/mL) | LOD 3 (pg/mL) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 08/31/15 | OTOL | Fluka | 1421704V | 10.720 | 1.0666 | 95.000 | 1.013 | 101.331 | 20.266 | 40.532 | 60.798 |
| 07/28/14 | 26DM | Aldrich | STBB5452V | 7.840 | 0.7722 | 130.000 | 1.004 | 100.391 | 20.078 | 40.156 | 60.235 |
| 07/28/14 | OANS | Fluka | SZBB343XV | 9.630 | 0.9601 | 105.000 | 1.008 | 100.812 | 20.162 | 40.325 | 60.487 |
| 08/31/15 | 1AMN | Fluka | SZB8268XV | 10.100 | 1.0090 | 100.000 | 1.009 | 100.899 | 20.180 | 40.360 | 60.539 |
| 08/31/15 | 2AMN | Sigma | SLBD0851V | 11.090 | 1.0868 | 90.000 | 0.978 | 97.814 | 19.563 | 39.126 | 58.688 |
| 07/28/14 | 4ABP | Aldrich | 128K1386V | 10.830 | 1.0722 | 90.000 | 0.965 | 96.495 | 19.299 | 38.598 | 57.897 |

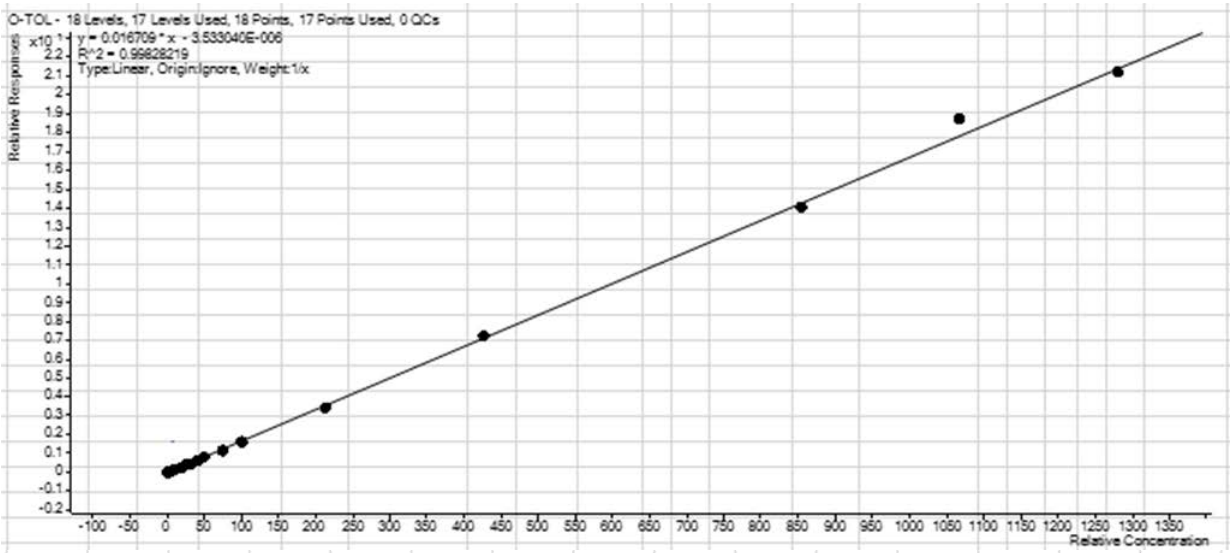
*Stock A was made in 10 mL ethanol.*
*Dilution C was made in 25 mL ethanol.*
*Dilution D was made in 100 mL ethanol.*
*Dilution E was made by diluting 1 ml of Dilution D to 10 mL of ethanol.*

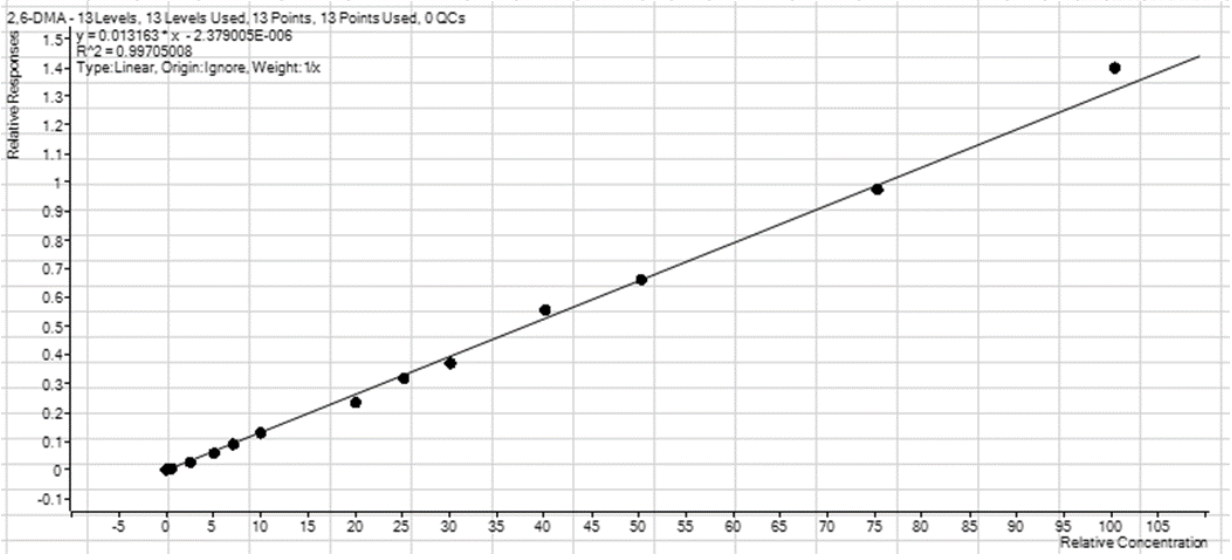
# Appendix B

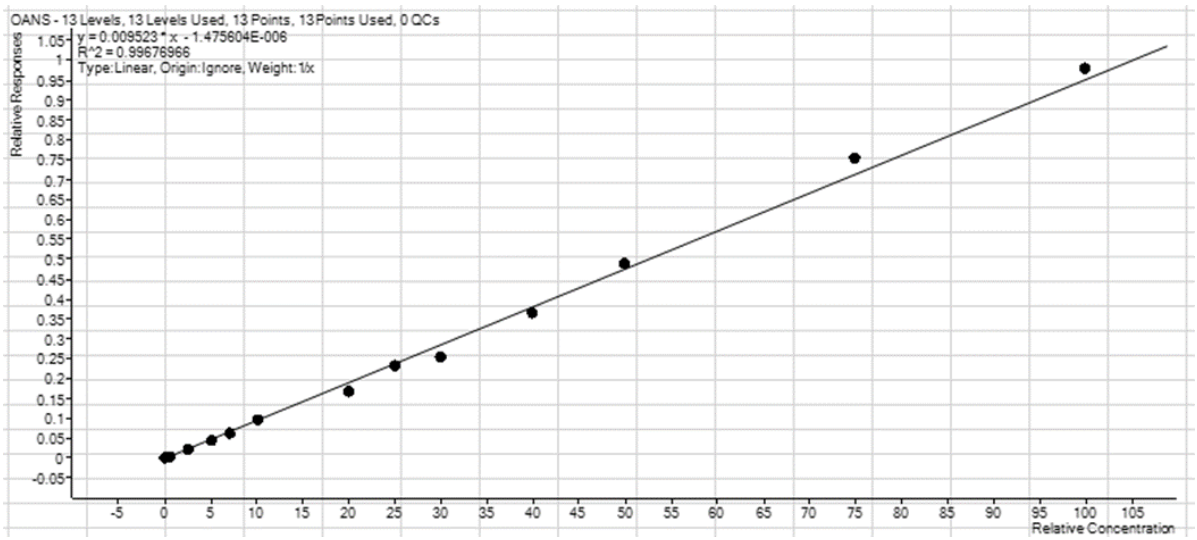
Method Validation Data

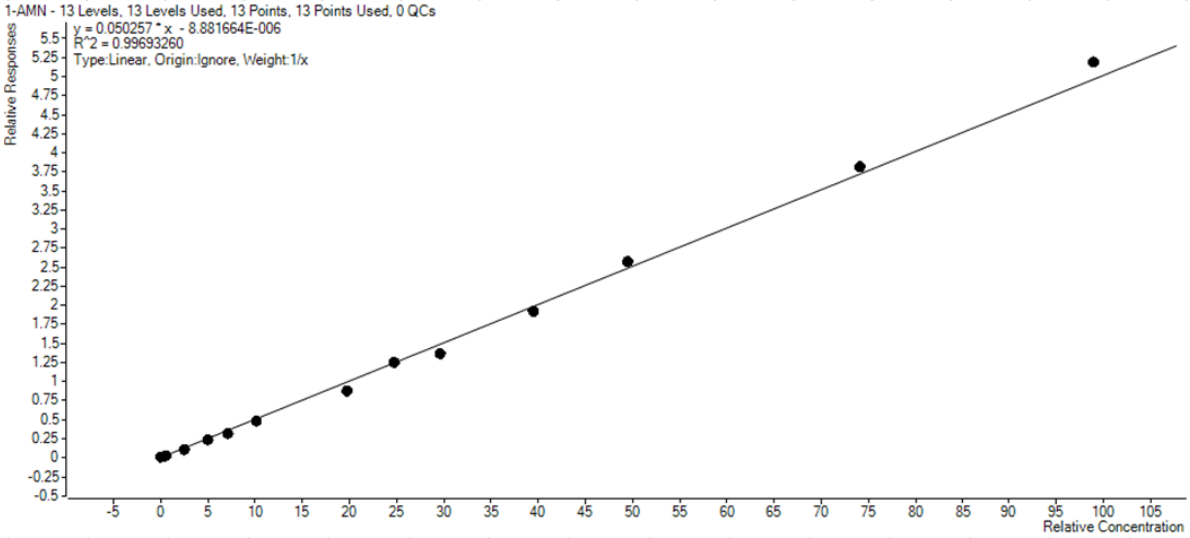
## *a. Linearity Limits*

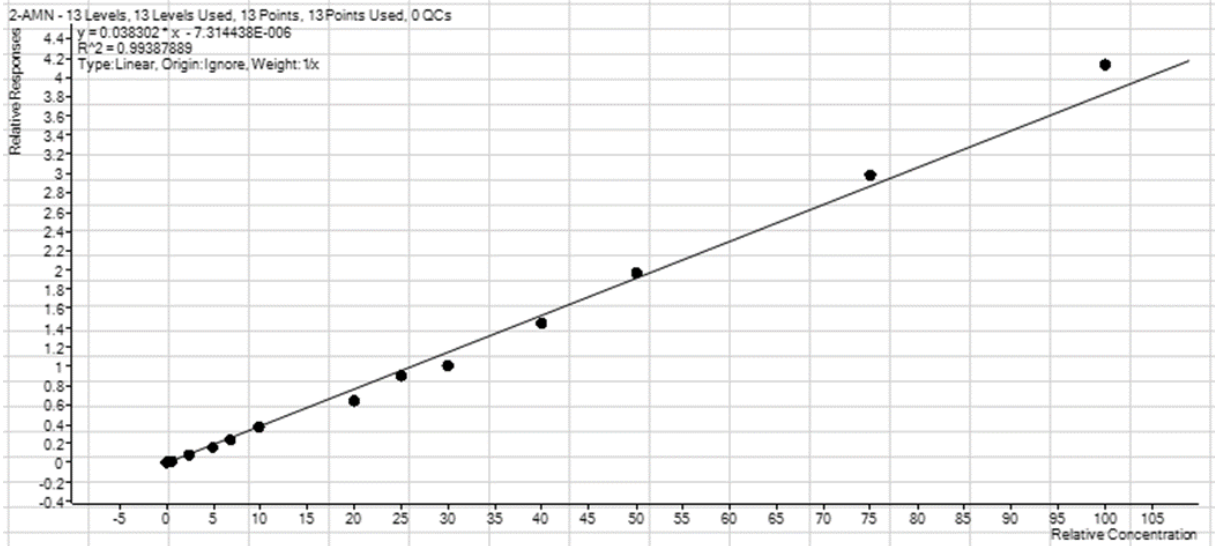
We have confirmed linear responses for all analytes across a broad range of analyte concentrations relevant to urinary levels of aromatic amines ($R^2 \geq 0.99$). As shown below, the linear range of the analytical method extends from the lowest to highest calibrator, 0.5 to 200 pg/µL for all analytes except OTOL, which extend to 1,200 pg/µL. Show below are calibration curves for all analytes.

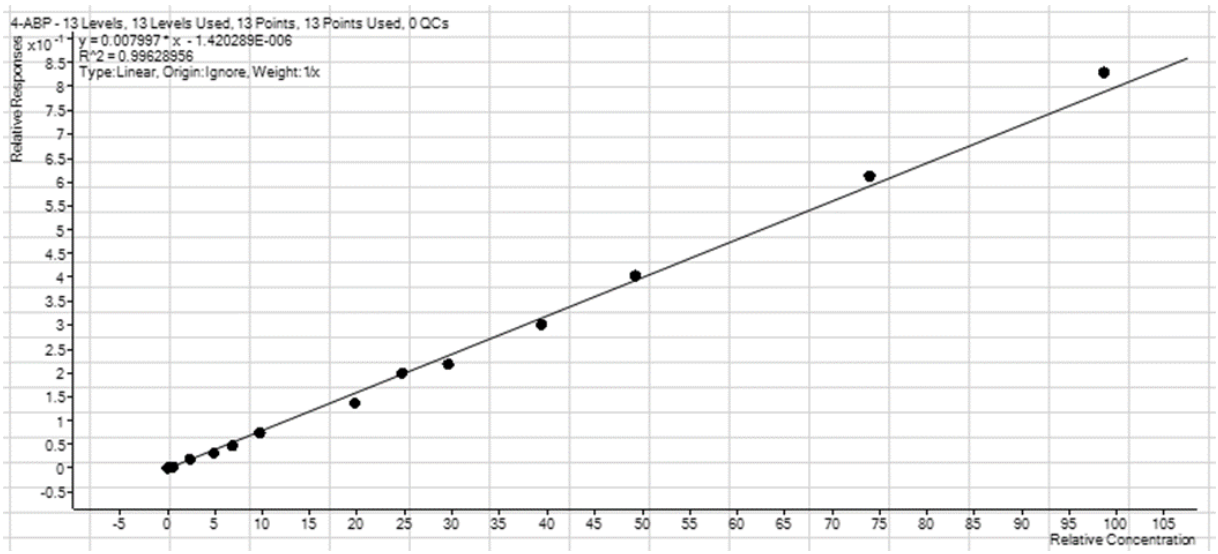


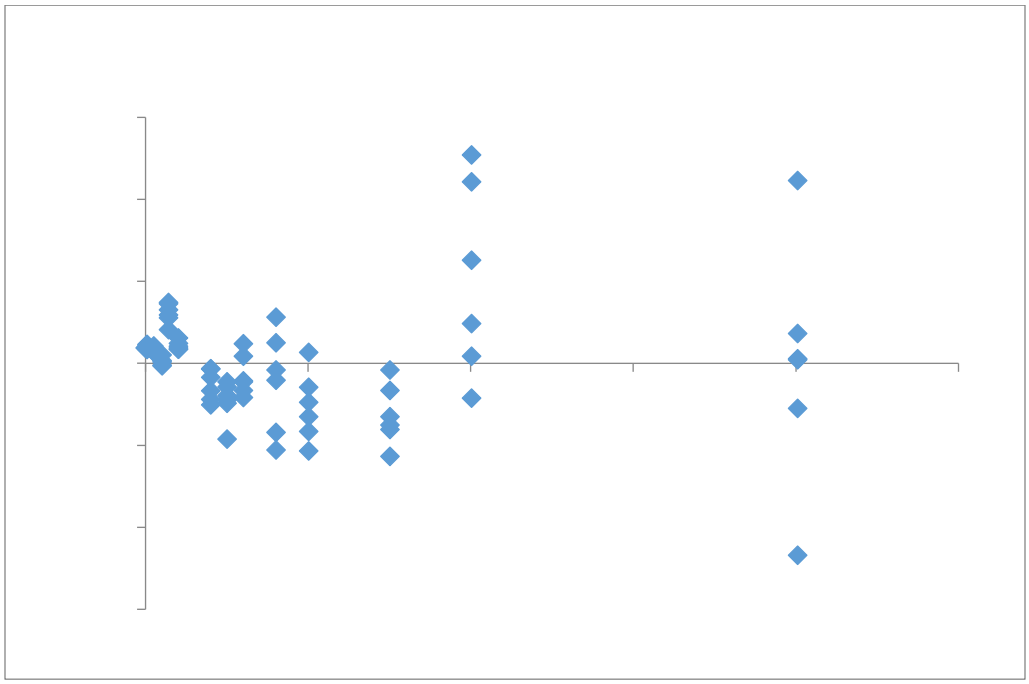

OANS - 13 Levels, 13 Levels Used, 13 Points, 13 Points Used, 0 QCs
y = 0.009523 * x - 1.475604E-006
R^2 = 0.99676966
Type:Linear, Origin:Ignore, Weight:1/x
Relative Responses
Relative Concentration

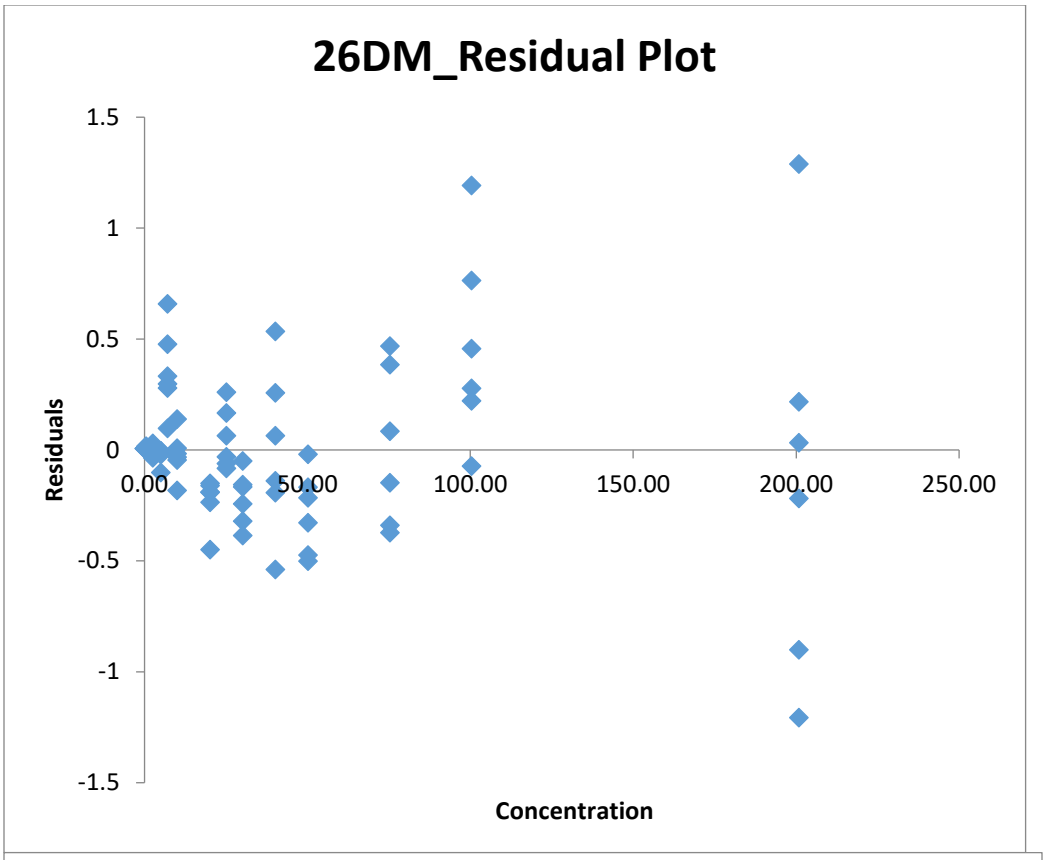

1-AMN - 13 Levels, 13 Levels Used, 13 Points, 13 Points Used, 0 QCs
y = 0.050257 * x - 8.881664E-006
R^2 = 0.99693260
Type:Linear, Origin:Ignore, Weight:1/x
Relative Responses
Relative Concentration

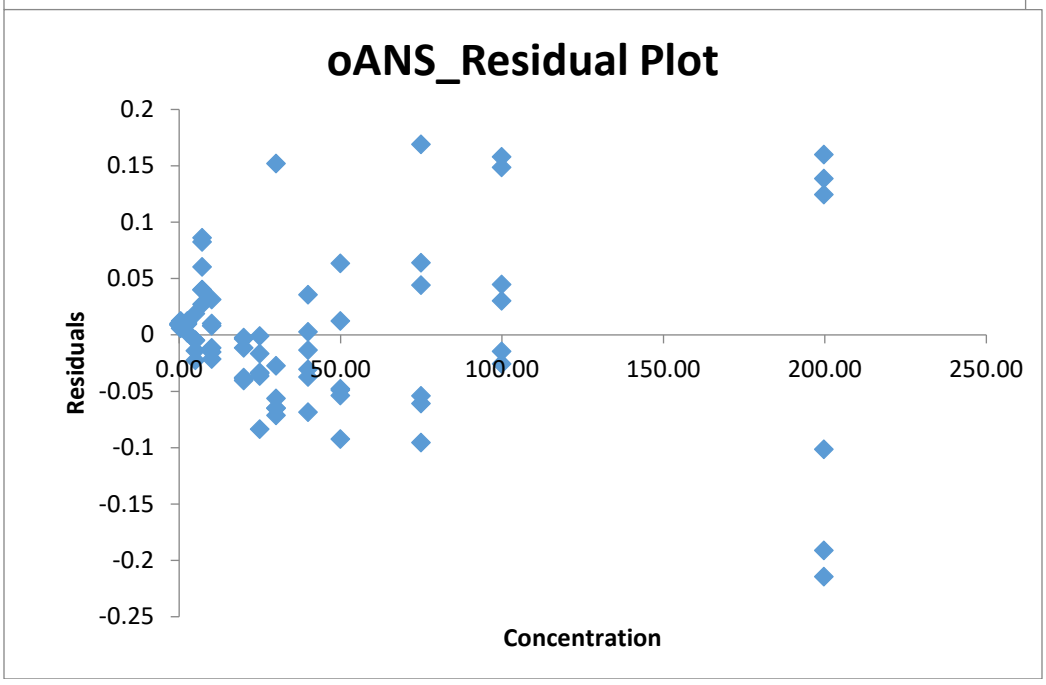

2-AMN - 13 Levels, 13 Levels Used, 13 Points, 13 Points Used, 0 QCs
y = 0.038302 * x - 7.314438E-006
R^2 = 0.99387889
Type:Linear, Origin:Ignore, Weight:1/x
Relative Responses
Relative Concentration

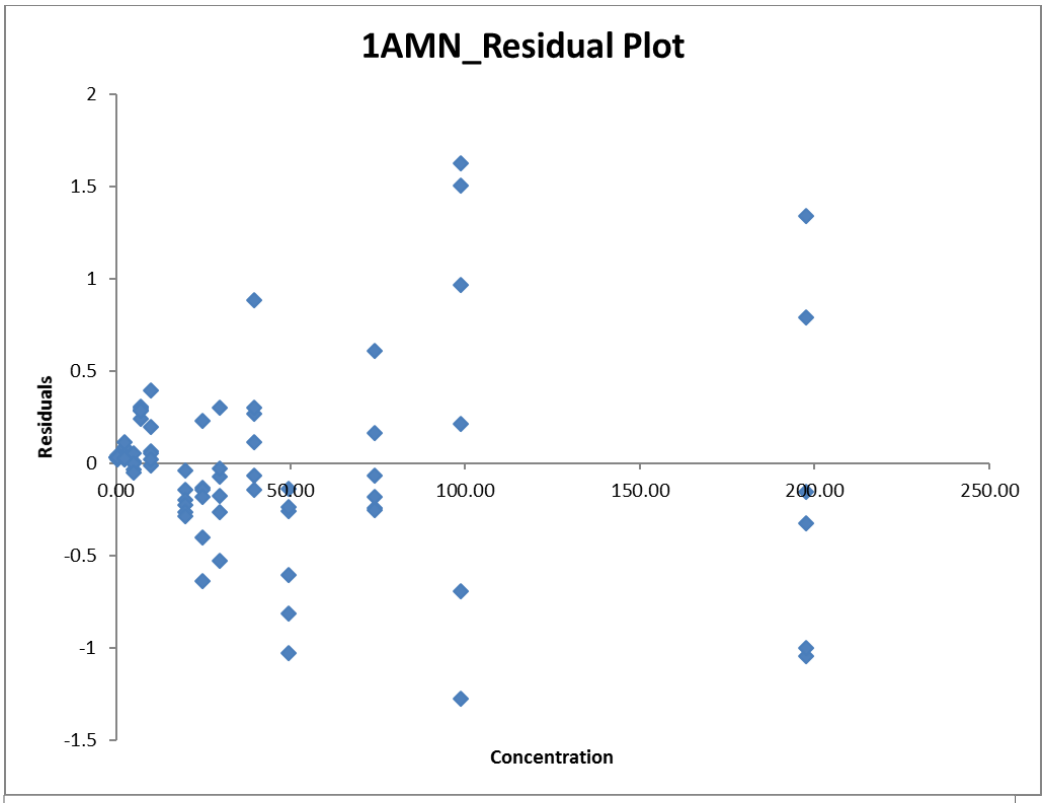


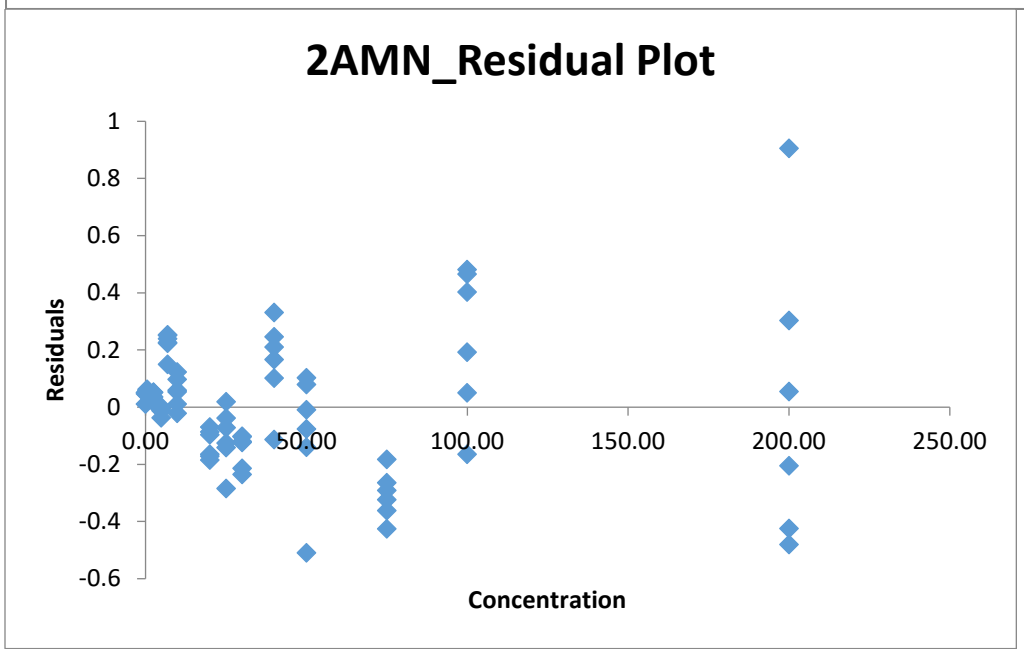

A weighting factor of 1/x is used for all analytes base on the residual plot for each analyte shown below.

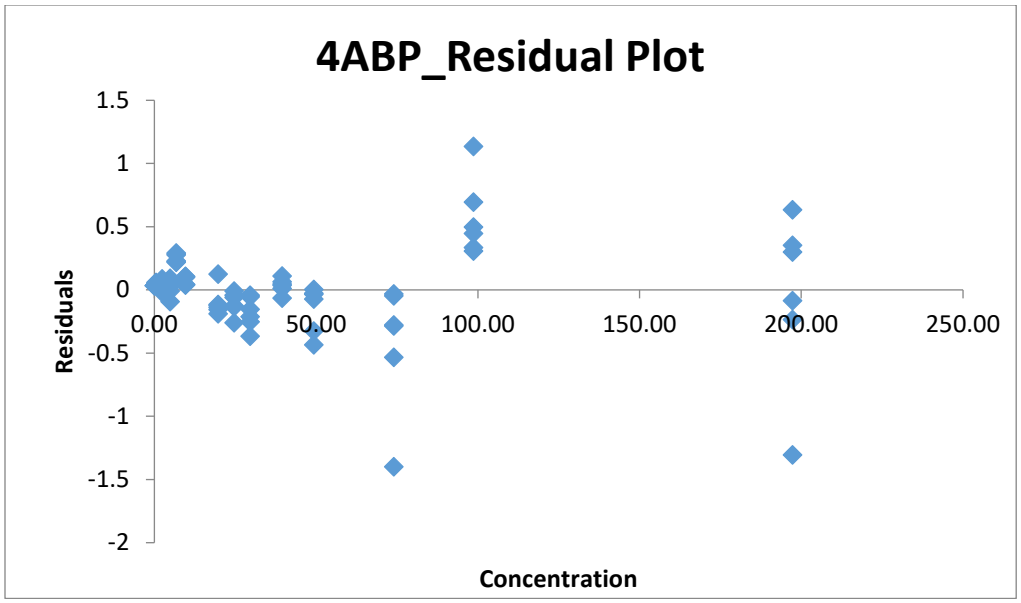



26DM_Residual Plot
1.5
1
0.5
0
-0.5
-1
-1.5
Residuals
0.00
50.00
100.00
150.00
200.00
250.00
Concentration


oANS_Residual Plot
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
Residuals
0.00
50.00
100.00
150.00
200.00
250.00
Concentration


1AMN_Residual Plot
Residuals
Concentration
2
1.5
1
0.5
0
-0.5
-1
-1.5
0.00
50.00
100.00
150.00
200.00
250.00


2AMN_Residual Plot
Residuals
Concentration
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
0.00
50.00
100.00
150.00
200.00
250.00



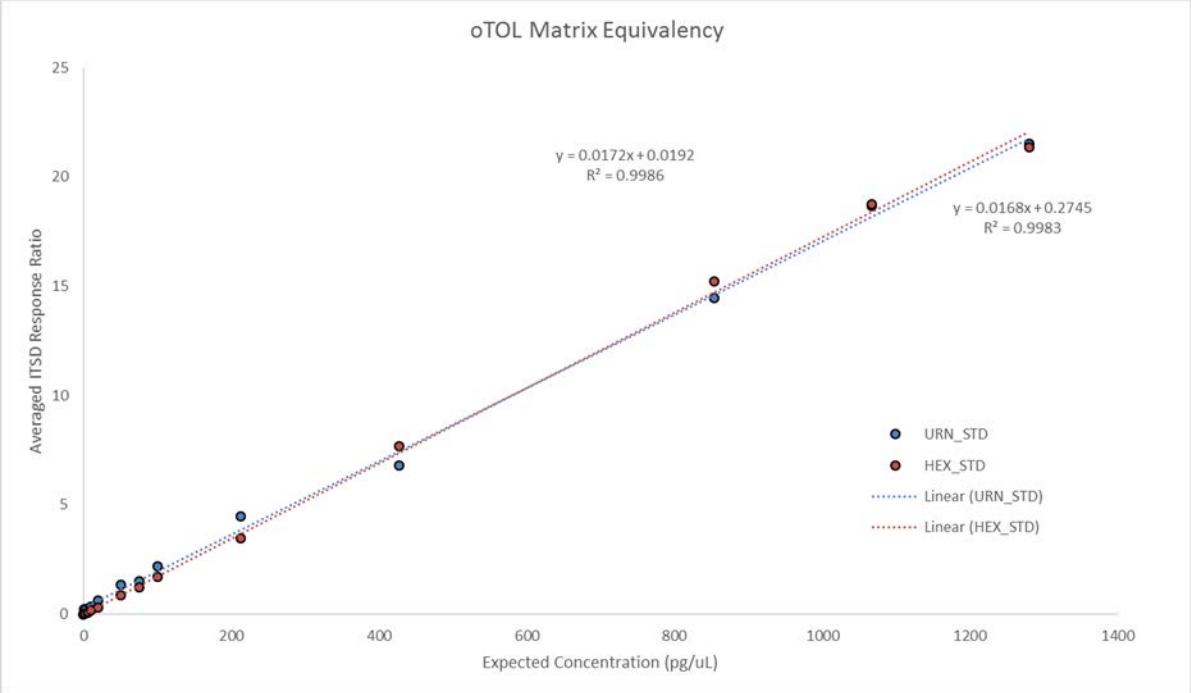

### *b. Calibration curve- Matrix vs non-matrix based calibrator*

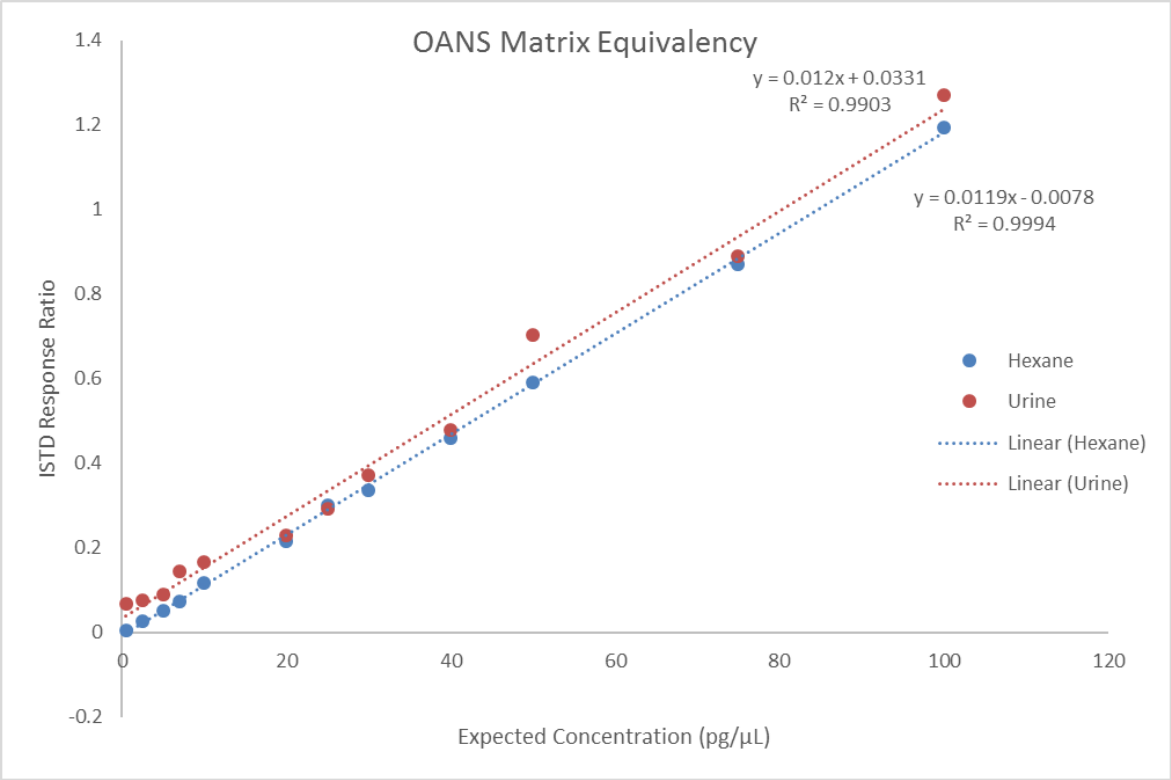
To verify matrix equivalency, 14 standard solutions were prepared as specified in Appendix A in hexane (non-matrix) and in urine (matrix) from the same stock solutions at the same time the hexane standards were made. The urine standards were carried through sample preparation as described in Section 8a for urine samples.

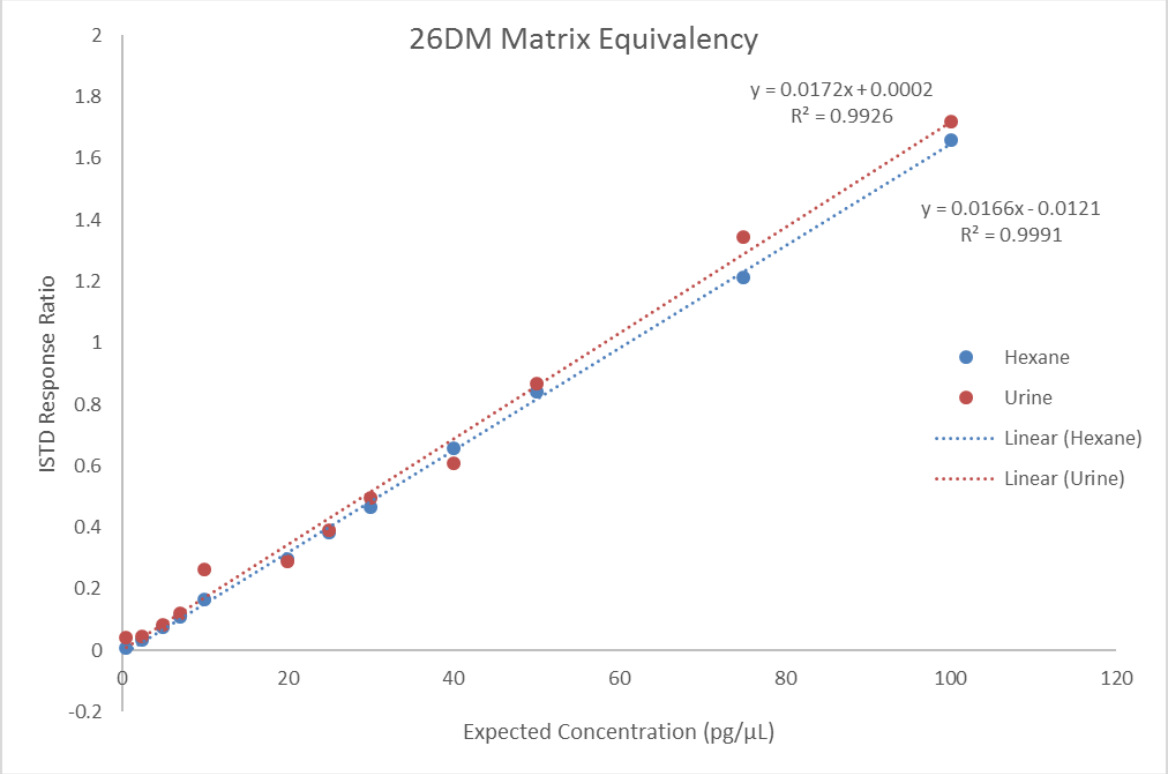
Each calibrator set was run 3 times, and the average of three slopes was obtained. The difference between the two average slopes of hexane and matrix set was less than 5% for all 5 analytes. The results indicate that there is no significant difference observed between the two calibration curves prepared in hexane or in urine.

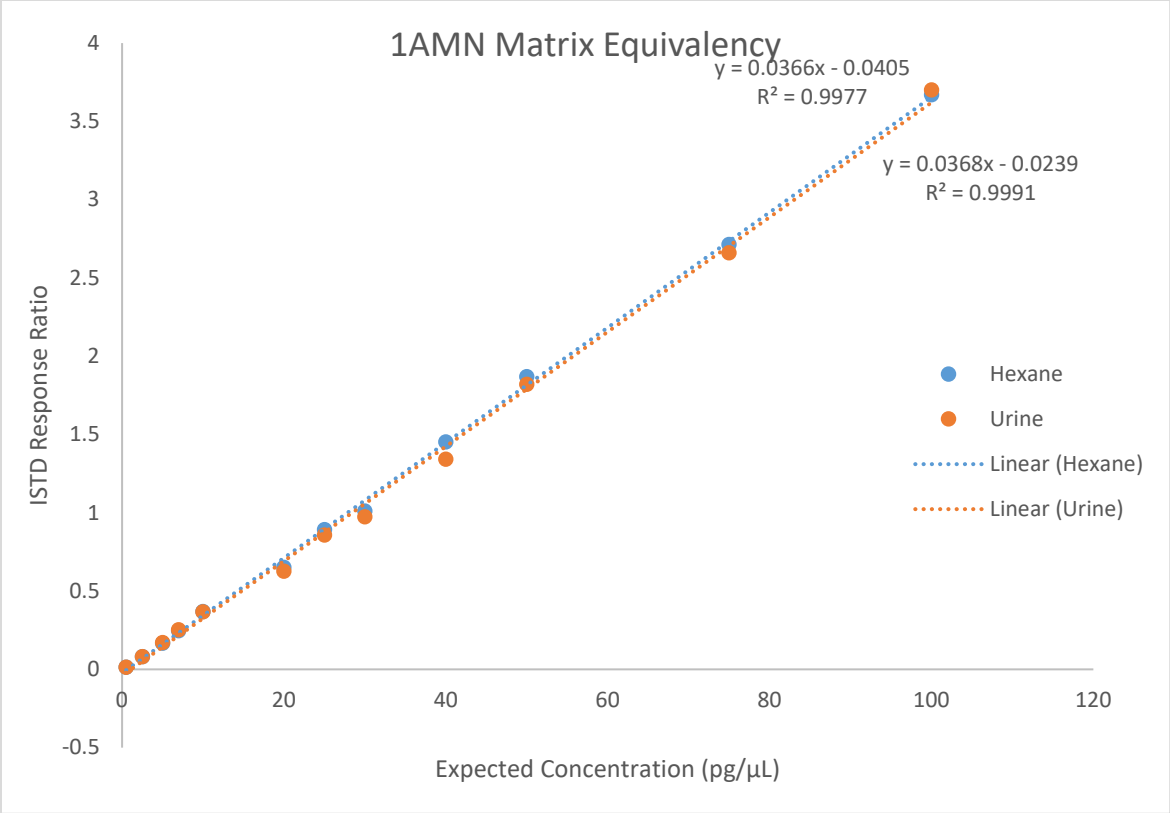
## Table B3:

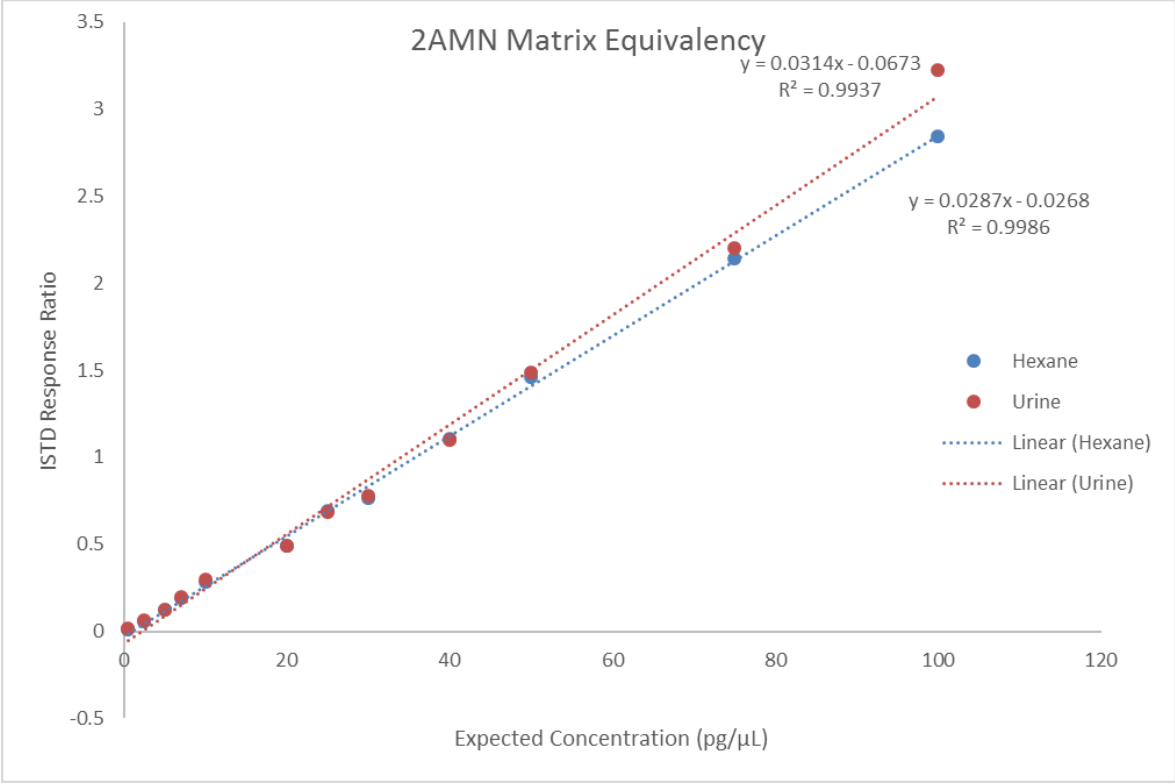
| Analyte | Hexane and urine calibration range (pg/µL) | Run date | Hexane calibration curve, slope | | | Hexane, averaged slopes | Urine calibration curve, slope | | | Urine, averaged slopes | Difference in averaged slopes (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Run 1 | Run 2 | Run 3 | | Run 1 | Run 2 | Run 3 | | |
| OTOL | 0.507 - 1280 | 11/6 - 11/9/17 | 0.01811 | 0.01673 | 0.01753 | 0.01746 | 0.01768 | 0.01688 | 0.01598 | 0.01685 | 3.49 |
| 26DM | 0.502 - 100 | 10/16 - 10/18/17 | 0.01613 | 0.01567 | 0.01563 | 0.01581 | 0.01643 | 0.01573 | 0.01611 | 0.01609 | -1.76 |
| OANS | 0.504 - 99.9 | 10/16 - 10/18/17 | 0.01164 | 0.01144 | 0.01160 | 0.01156 | 0.01153 | 0.01099 | 0.01141 | 0.01131 | 2.19 |
| 1AMN | 0.504 - 99.9 | 10/16 - 10/18/17 | 0.03636 | 0.03736 | 0.03735 | 0.03702 | 0.03553 | 0.03610 | 0.03574 | 0.03579 | 3.33 |
| 2AMN | 0.489 - 100 | 10/16 - 10/18/17 | 0.02788 | 0.02850 | 0.02849 | 0.02829 | 0.02924 | 0.02948 | 0.02924 | 0.02932 | -3.64 |
| 4ABP | 0.482 - 98.6 | 10/16 - 10/18/17 | 0.00699 | 0.00698 | 0.00673 | 0.00690 | 0.00712 | 0.00716 | 0.00697 | 0.00708 | -2.62 |

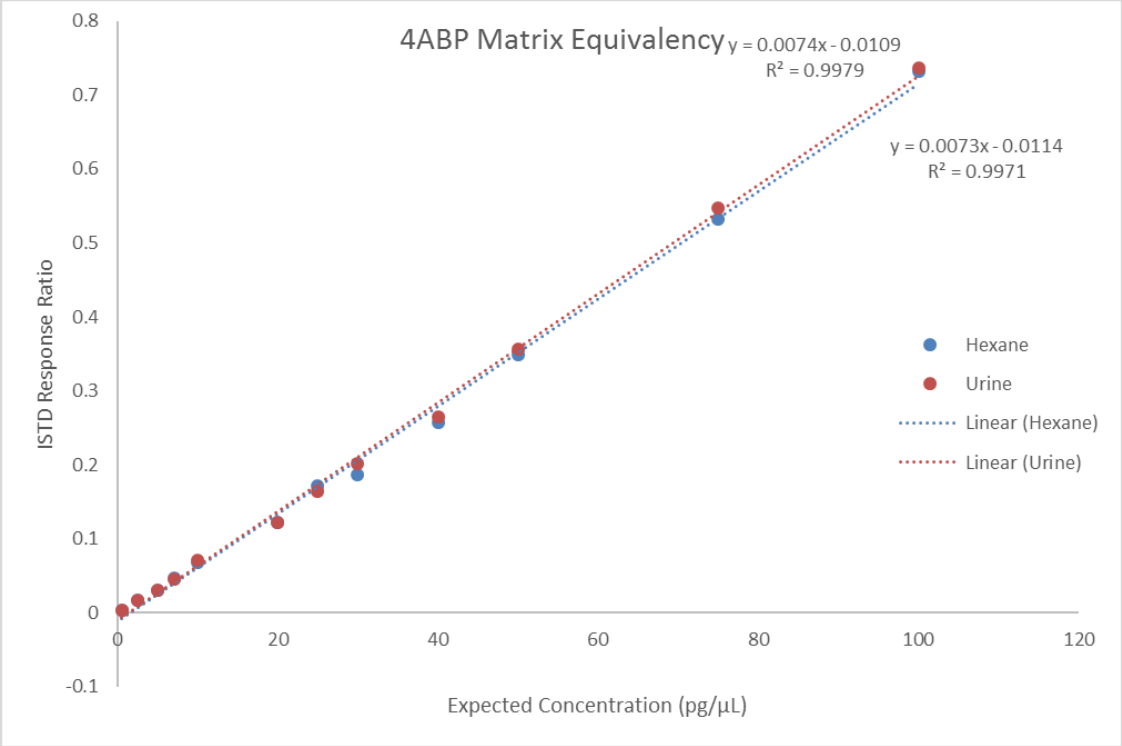



OANS Matrix Equivalency
y = 0.012x + 0.0331
R² = 0.9903
y = 0.0119x - 0.0078
R² = 0.9994
Hexane
Urine
Linear (Hexane)
Linear (Urine)
ISTD Response Ratio
Expected Concentration (pg/µL)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
20
40
60
80
100
120


26DM Matrix Equivalency
y = 0.0172x + 0.0002
R² = 0.9926
y = 0.0166x - 0.0121
R² = 0.9991
Hexane
Urine
Linear (Hexane)
Linear (Urine)
ISTD Response Ratio
Expected Concentration (pg/µL)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
20
40
60
80
100
120


1AMN Matrix Equivalency
y = 0.0366x - 0.0405
R² = 0.9977
y = 0.0368x - 0.0239
R² = 0.9991
ISTD Response Ratio
Expected Concentration (pg/µL)
Hexane
Urine
Linear (Hexane)
Linear (Urine)
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
0
20
40
60
80
100
120


2AMN Matrix Equivalency
y = 0.0314x - 0.0673
R² = 0.9937
y = 0.0287x - 0.0268
R² = 0.9986
ISTD Response Ratio
Expected Concentration (pg/µL)
Hexane
Urine
Linear (Hexane)
Linear (Urine)
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
0
20
40
60
80
100
120




### *c. Limit of Detection*

The method detection limits for AAs in human samples are determined according to the guideline for determination of limits of detection by the Clinical and Laboratory Standard Institute (CLSI. Protocols for Determination of Limits of Detection and Limits of Quantitation: Approved Guideline. CLSI document EP17-A. Wayne, PA: Clinical and Laboratory Standards Institute, 2004). Four urine pools: one non-smoker blank pool (LoD0), non-smoker blank spiked approximately at 20, 40, and 60 pg/mL (LoD1, LoD2, and LoD3 respectively) for 6 analytes: *o*-toluidine, 2,6-dimethylaniline, *o*-anisidine, 1-aminonaphthalene, 2-aminonaphthalene, and 4-aminobiphenyl. Preparing details are in Appendix A, Section B2. These four pools are being used to estimate the LoDs. LoDs were obtained from 50 independent runs, and the results are listed below.

Table B4:

| Analytes | LOD (pg/mL) |
|---|---|
| OTOL | 111.22 |
| 26DM | 15.67 |
| OANS | 7.02 |
| 1AMN | 1.29 |
| 2AMN | 2.79 |
| 4ABP | 1.75 |

## c. Accuracy

All native compounds were purchased from Sigma and affiliates and used to prepare calibration curve. Native standards purchased from a different vendor, mostly TRC, were used to prepared accuracy testing solutions.
Accuracy was determined by spiking known amounts of AA standard solution into hexane (accuracy in solution) and urine (accuracy in matrix). The spiked urine standards were prepared the same procedure as for unknown samples.

*%bias =100* (observed AA level-expected AA level)/expected AA level*

Accuracy in solution- All analytes used to prepare the calibration curves and testing calibrators were purchased from different manufactures as listed in table below. If a different vendor was not available, a different lot was purchased from the same vendor.

Accuracy testing solutions were run with a hexane calibration curve, and the results are shown below.

Table B5: Accuracy in solution (%bias)

| | %-bias | | | | |
|---|---|---|---|---|---|
| Analyte | ACC-1 | ACC-2 | ACC-3 | ACC-4 | ACC-5 |
| OTOL | -7.43 | -22.71 | -2.22 | -1.63 | -7.48 |
| 26DM | 3.78 | 1.84 | 1.80 | -1.03 | 0.13 |
| OANS | -13.19 | 4.86 | -7.44 | -12.39 | -16.53 |
| 1AMN | -4.83 | -1.25 | 2.55 | -12.24 | -3.52 |
| 2AMN | 5.28 | 6.54 | 2.55 | 5.18 | 0.12 |
| 4ABP | -9.06 | 0.15 | 11.84 | -8.43 | -14.83 |

Accuracy in matrix was tested with three separate runs in three days, at three levels, and in triplicate at each level. All %bias except one are less than 15%, and accuracy in matrix tests are acceptable.

Table B6: Accuracy in matrix (%bias)

| Analyte | Expected Conc (pg/mL) | Avg Calc Conc (pg/mL) | %-bias |
|---|---|---|---|
| OTOL | 506.66 | 478.08 | 5.64 |
| | 101.33 | 96.77 | 4.50 |
| | 50.67 | 56.35 | -11.23 |
| 26DM | 506.96 | 524.09 | -3.38 |
| | 152.09 | 165.04 | -8.52 |
| | 50.70 | 54.72 | -7.94 |
| OANS | 509.06 | 545.71 | -7.20 |
| | 152.72 | 159.90 | -4.70 |
| | 50.91 | 57.11 | -12.19 |
| 1AMN | 110.76 | 108.13 | 2.37 |
| | 106.61 | 94.51 | 11.35 |
| | 458.10 | 457.46 | 0.14 |
| 2AMN | 489.07 | 525.85 | -7.52 |
| | 146.72 | 160.45 | -9.36 |
| | 48.91 | 53.46 | -9.30 |
| 4ABP | 109.03 | 103.12 | 5.42 |
| | 109.17 | 104.17 | 4.58 |
| | 349.32 | 321.83 | 7.87 |

## d. Precision

Several pools were used to determine precision of our aromatic amines assay. The QC pools were prepared in house from urine collected from non-smokers and spiked with native standard solution (Appendix A, Section B.2). The QCs samples were prepared and analyzed. The CV% values for intra-day (n=5) and inter-day (n=5) are calculated and listed in the table below. All CV% values are less than 10% and thus acceptable.

Table B7: Inter and intra-day precision (n = 5)

| | | Intra-day (N=5) | | Inter-day (N=5) | |
|---|---|---|---|---|---|
| Analyte | Sample ID | Avg Measured Conc (pg/mL) | CV% | Avg Measured Conc (pg/mL) | CV% |
| OTOL | QC_Low2016 | 188.5 | 7.73 | 185.4 | 5.60 |
| | QC_Low2015 | 164.5 | 5.40 | 159.8 | 6.26 |
| | QCHigh_2016 | 448.8 | 4.74 | 461.3 | 5.18 |
| | QCHigh_2015 | 503.2 | 5.55 | 527.3 | 5.75 |
| 26DM | QC_Low2016 | 137.4 | 2.82 | 140.8 | 3.60 |
| | QC_Low2015 | 113.9 | 6.22 | 115.6 | 5.88 |
| | QCHigh_2016 | 410.5 | 3.97 | 421.4 | 5.61 |
| | QCHigh_2015 | 487.1 | 3.50 | 503.0 | 4.37 |
| OANS | QC_Low2016 | 140.6 | 2.14 | 145.3 | 1.84 |
| | QC_Low2015 | 149.5 | 3.61 | 157.9 | 2.62 |
| | QCHigh_2016 | 385.6 | 3.94 | 410.5 | 1.38 |
| | QCHigh_2015 | 506.5 | 2.78 | 526.1 | 3.44 |
| 1AMN | QC_Low2016 | 108.5 | 2.35 | 109.8 | 3.37 |
| | QC_Low2015 | 120.0 | 2.19 | 118.2 | 4.48 |
| | QCHigh_2016 | 309.1 | 3.14 | 310.9 | 2.89 |
| | QCHigh_2015 | 491.9 | 2.19 | 492.8 | 3.34 |
| 2AMN | QC_Low2016 | 102.3 | 1.93 | 105.7 | 1.97 |
| | QC_Low2015 | 116.7 | 1.38 | 121.0 | 4.31 |
| | QCHigh_2016 | 314.3 | 2.11 | 331.4 | 5.45 |
| | QCHigh_2015 | 505.6 | 2.44 | 524.4 | 3.84 |
| 4ABP | QC_Low2016 | 108.5 | 1.76 | 113.6 | 2.99 |
| | QC_Low2015 | 112.7 | 2.51 | 116.5 | 5.12 |
| | QCHigh_2016 | 345.4 | 2.96 | 366.5 | 3.22 |
| | QCHigh_2015 | 468.3 | 1.13 | 479.1 | 3.31 |

*Matrix blank contained oTOL, oANS, and 2AMN.*

### f. Carry over

Carryover was examined by comparing successive pairs of injections of the highest calibrator, 200 pg/µl (ng/ml) or high QC samples followed by solvent blank, toluene. No carry over was observed in the solvent blank after any injection of the highest calibrator or QC. As a precaution, a toluene blank injection is done followed a calibration curve, QC high samples, and an analytical run. Between each individual injection, the injection syringe is washed with 6x3 µl toluene (injection volume is 1 µl).

### g. Thaw-refreeze and storage stability

Samples are shipped frozen and stored at -70°C±10°C until processed (thawed and aliquoted) or prepared. Residual samples are re-frozen and stored at -70°C±10°C. Samples might be thawed again if repeated analysis is needed.

Repeated thaw and re-freeze cycles were carried out, and the results indicate that all analytes were stable following at least 5 thaw-refreeze cycles (T/RF) (sample loss ≤ 5-10%). Results for individual analytes are listed below.

Table B8: Freeze/thaw stability after 5 cycles

| Analyte | Control Sample Conc (pg/mL) | %-bias after five T/RF cycles |
|---|---|---|
| OTOL | 218.0 | 9.09 |
| 26DM | 157.5 | 5.46 |
| OANS | 158.2 | 8.71 |
| 1AMN | 109.5 | 16.39 |
| 2AMN | 101.4 | 10.93 |
| 4ABP | 111.6 | 5.75 |

Prepared urine samples are routinely stored at approximately -20ºC±4°C until they are analyzed on a GS/MS-MS system. During GC/MS-MS analysis, samples are stored in a cooled sample drawer at approximately 10°C. Results from repeated injections of samples stored in the cool tray at 10°C indicated that all analytes levels are not be significantly impacted by this short term storage conditions: 24 hour (overnight) after the first injection and 72 hours after the first injection (over a weekend). These conditions were chosen to ensure the longest time (that a sample would be left in the cool tray before being taken out and stored again) were tested.

Table B9: Short-term storage stability at 10°C

| Analyte | Day 1 Conc (pg/mL) | %-bias to Day 1 in 24 hrs | %-bias to Day 1 in 72 hrs |
|---|---|---|---|
| OTOL | 623.0 | 5.53 | 9.66 |
| 26DM | 459.6 | 7.11 | 8.98 |
| OANS | 427.1 | 1.60 | 5.15 |
| 1AMN | 269.2 | -6.87 | -12.71 |
| 2AMN | 318.8 | -0.81 | -1.54 |
| 4ABP | 347.0 | 2.06 | 1.46 |

Table B10: Short-term storage stability at -20°C

| Analyte | Day 1 Conc (pg/mL) | %-bias to Day 1 in one week | %-bias to Day 1 in 13 weeks |
|---|---|---|---|
| OTOL | 57.0 | -6.55 | 6.72 |
| | 67.6 | 11.61 | 5.86 |
| | 82.2 | -6.38 | -12.25 |
| | 168.0 | -0.40 | -7.79 |
| | 490.2 | -1.75 | -2.26 |
| 26DM | 19.8 | 10.28 | 10.92 |
| | 43.8 | 1.95 | 13.38 |
| | 68.1 | 9.89 | 12.45 |
| | 149.6 | 1.57 | -0.63 |
| | 543.4 | 11.58 | 8.16 |
| OANS | 44.9 | -3.25 | -20.39 |
| | 70.5 | 5.88 | 9.05 |
| | 92.8 | 10.51 | 6.87 |
| | 153.5 | 10.39 | 7.81 |
| | 544.4 | 2.28 | 5.14 |
| 1AMN | 13.6 | -14.37 | -3.62 |
| | 34.1 | 1.30 | 6.84 |
| | 43.8 | -21.19 | -15.84 |
| | 94.1 | -22.87 | -21.25 |
| | 449.4 | -5.08 | -11.64 |
| 2AMN | 14.3 | -5.54 | -6.70 |
| | 35.4 | -7.76 | -6.46 |
| | 53.4 | -6.35 | -6.27 |
| | 96.1 | -4.72 | -3.42 |
| | 528.8 | 2.93 | 3.71 |
| 4ABP | 21.5 | 5.36 | -0.32 |
| | 42.5 | 2.65 | 0.90 |
| | 62.4 | 6.43 | 2.17 |
| | 120.2 | 11.81 | 10.04 |
| | 476.0 | 3.15 | 0.00 |

Table B11: Long-term stability of un-processed QC samples stored at -70°C for 2 years

| Analyte | Nominal Conc (pg/mL) | %-bias |
|---|---|---|
| OTOL | 485.9 | -1.48 |
| 26DM | 502.6 | 2.01 |
| OANS | 505.8 | 1.44 |
| 1AMN | 458.1 | -8.36 |
| 2AMN | 479.8 | -6.62 |
| 4ABP | 446.1 | -4.80 |

Table B12: Short-term stability for length of time needed to handle un-processed samples.

| Analyte | Nominal Conc (pg/mL) | %-bias |
|---|---|---|
| OTOL | 485.9 | -5.24 |
| 26DM | 502.6 | -3.27 |
| OANS | 505.8 | 3.37 |
| 1AMN | 458.1 | 5.08 |
| 2AMN | 479.8 | 0.63 |
| 4ABP | 446.1 | 3.99 |

## h. Ruggedness test

**QC AND UNKNOWN SAMPLES WERE USED IN RUGGEDNESS TEST BY VARYING THE FOLLOWING PARAMETERS:**

i. Injection Pulse Pressure: 25 psi, 30 psi (final method condition), and 35 psi. This could happen occasionally if the carry gas, He, is not pressurized enough during injection time. Injection pressure plays an important role on how much analyte being pushed on to analytical column.
ii. Injection Port Temperature: 240ºC, 250 ºC (final method condition), and 260 ºC. The injection port temperature could be varied slightly due to the insulator or heating element around the injection port. The injection port temperature affect the evaporation of injected sample, thus overall assay sensitivity.
iii. Source temperature: 270ºC, 280 ºC (final method condition), and 290 ºC. The source temperature could be varied slightly due to the flow of the GC column. Source temperature could affect the ionization of the analytes, thus the overall sensitivity of the assay.
iv. Amount of PFPA used during derivatization: 2 µl, 3 µl (final method condition), 4 µl. The amount of PFPA has a crucial role in the efficiency of the assay derivatization.

v. GC analytical column lots (country it was made, year it was made, the work order/polymer lot or batch, the run of the work order, the column of the work order, internal notification): GC column performance plays a crucial roles in the overall data quality: chromatographic peak, sensitivity and thus quantification of each final result. This test was done differently than other parameters (no %different between tested and final method condition). The same samples were injected on different columns of different lots, and the % different was calculated and listed. There were 3 lots tested, including one special order. The other 2 lots were spanned approximately one year.

Table B13: Ruggedness test results (%different between tested and final method condition)

| Analyte | Measured Conc (pg/mL) at 250°C inlet (control) | 240°C:250°C %-bias | 260°C:250°C %-bias |
|---|---|---|---|
| OTOL | 214.7 | -2.02 | 5.27 |
| 26DM | 150.7 | 1.02 | -2.65 |
| OANS | 133.6 | -4.33 | -3.49 |
| 1AMN | 97.9 | -9.15 | -0.71 |
| 2AMN | 93.0 | -9.37 | 1.89 |
| 4ABP | 105.6 | -13.20 | -6.91 |

| Analyte | Measured Conc (pg/mL) at 30 psi pulsed pressure (control) | 25psi:30psi %-bias | 35psi:30psi %-bias |
|---|---|---|---|
| OTOL | 214.7 | 15.61 | -0.20 |
| 26DM | 150.7 | -3.67 | -1.09 |
| OANS | 133.6 | -5.81 | -13.27 |
| 1AMN | 97.9 | -0.96 | -2.87 |
| 2AMN | 93.0 | 1.08 | 0.07 |
| 4ABP | 105.6 | -8.10 | -12.17 |

| Analyte | Measured Conc (pg/mL) with 3 µL PFPA (control) | 2µL:3µL %-bias | 4µL:3µL %-bias |
|---|---|---|---|
| OTOL | 448.1 | -15.12 | 1.40 |
| 26DM | 456.0 | 9.23 | 7.98 |
| OANS | 403.4 | 2.86 | 7.00 |
| 1AMN | 314.9 | 6.21 | 1.02 |
| 2AMN | 315.8 | 2.00 | 2.43 |
| 4ABP | 337.0 | 0.32 | 0.78 |

| Analyte | Measured Conc (pg/mL) with 15hr hydrolysis (control) | 14hr:15hr %-bias | 16hr:15hr %-bias |
|---|---|---|---|
| OTOL | 448.1 | -2.73 | -14.49 |
| 26DM | 456.0 | 4.60 | 9.74 |
| OANS | 403.4 | 1.20 | 3.52 |
| 1AMN | 314.9 | -1.39 | 8.64 |
| 2AMN | 315.8 | 0.95 | 1.44 |
| 4ABP | 337.0 | -2.26 | 0.38 |

| Urine samples tested | OTOL Lot #1:Lot #2, %-bias | 26DM Lot #1:Lot #2, %-bias | OANS Lot #1:Lot #2, %-bias | 1AMN Lot #1:Lot #2, %-bias | 2AMN Lot #1:Lot #2, %-bias | 4ABP Lot #1:Lot #2, %-bias |
|---|---|---|---|---|---|---|
| Sample 1 | -12.59 | 10.68 | 7.36 | 1.05 | -0.78 | -2.22 |
| Sample 2 | 4.54 | -0.21 | -2.16 | 1.09 | -0.22 | -1.02 |
| Sample 3 | -1.64 | -11.54 | 1.72 | 7.80 | 3.16 | 2.56 |
| Sample 4 | 11.94 | -2.67 | -3.41 | 3.79 | 0.51 | 1.73 |
| Sample 5 | 3.07 | -4.01 | -0.67 | 13.74 | 6.16 | 4.79 |
| Sample 6 | 1.16 | -5.13 | 0.45 | 8.81 | 1.19 | 1.72 |
| Sample 7 | 12.78 | -12.72 | -0.13 | 6.46 | 2.62 | -1.49 |
| Sample 8 | -4.92 | -9.90 | 3.89 | 2.52 | 1.78 | 2.17 |
| Sample 9 | -7.84 | -0.75 | -1.36 | 9.28 | 1.54 | -0.24 |
| Sample 10 | -0.42 | 2.21 | -3.49 | 3.45 | 0.66 | -0.08 |
| Sample 11 | -6.17 | -9.94 | -2.83 | 7.23 | -1.86 | 5.45 |
| Sample 12 | -2.91 | -0.56 | -2.87 | 0.28 | 3.82 | 0.42 |
| Sample 13 | 4.08 | -2.78 | 4.40 | 4.44 | -2.44 | 0.13 |
| Sample 14 | -7.27 | 1.34 | 1.10 | 2.39 | 4.18 | 3.72 |
| Sample 15 | 0.42 | -15.33 | 4.49 | 0.78 | 3.71 | -11.10 |
| Sample 16 | 10.68 | -0.76 | 2.29 | 6.02 | -0.30 | 0.02 |

| | OTOL | 26DM | OANS | 1AMN | 2AMN | 4ABP |
|---|---|---|---|---|---|---|
| Urine samples tested | Lot #1:Lot #3, %-bias | Lot #1:Lot #3, %-bias | Lot #1:Lot #3, %-bias | Lot #1:Lot #3, %-bias | Lot #1:Lot #3, %-bias | Lot #1:Lot #3, %-bias |
| Sample 17 | 12.61 | -9.02 | -4.67 | 6.98 | -0.24 | -6.86 |
| Sample 18 | 16.36 | -3.12 | 2.54 | 6.51 | 0.07 | -5.98 |
| Sample 19 | 16.33 | 5.74 | 8.13 | 7.09 | -16.65 | -16.30 |
| Sample 20 | -14.85 | -0.03 | 0.68 | 7.01 | -2.18 | -6.37 |
| Sample 21 | -7.09 | -8.38 | -10.71 | 16.07 | -7.54 | -4.61 |
| Sample 22 | 1.97 | -5.85 | 4.52 | 12.22 | 0.62 | -0.71 |
| Sample 23 | 18.66 | -3.37 | 6.80 | -10.32 | -7.83 | 0.63 |
| Sample 24 | -21.82 | -2.87 | 1.17 | 8.51 | -9.39 | -6.92 |
| Sample 25 | 8.38 | -2.22 | -1.44 | 20.45 | -5.99 | -7.07 |
| Sample 26 | 1.31 | -6.26 | 6.46 | 7.47 | -1.91 | -1.51 |
| Sample 27 | 20.14 | -1.64 | -3.48 | -1.06 | -3.44 | 9.40 |
| Sample 28 | 9.83 | -4.40 | 2.24 | 5.27 | 5.31 | -7.42 |
| Sample 29 | -0.49 | -11.39 | 0.35 | 4.26 | 1.97 | -3.96 |
| Sample 30 | -14.65 | -10.20 | -3.50 | -3.01 | -4.60 | -16.75 |
| Sample 31 | -5.62 | -6.70 | -1.51 | 0.97 | 3.90 | -5.75 |
| Sample 32 | -14.46 | -4.36 | -1.90 | 9.78 | 2.69 | -0.14 |

## i. Instrument cross validation

There are 2 instruments used to analyze prepared samples, both are Agilent QQQ 7000C GC/MS-MS named Gonzo (main) and Animal (back up) respectively. 5 smokers and 5 nonsmokers were used for instrument cross validation.

| Analyte | Pearson Value |
|---|---|
| OTOL | 0.9782 |
| 26DM | 0.9716 |
| OANS | 0.9985 |
| 1AMN | 0.9993 |
| 2AMN | 0.9998 |
| 4ABP | 0.9998 |

# Appendix C

## Method Performance Documentation

Method performance documentation for this method including accuracy, precision, specificity, and stability is provided in this appendix. **The signatures of the Branch Chief and Director of the Division of the Laboratory Sciences on the first page of this procedure denote that the method performance is fit for the intended use of the method.**

**Accuracy using Spike Recovery**

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: Aromatic amines in human urine.
Method #: 2020.02
Matrix: **Urine**
Units: pg/mL
Analyte: **OTOL**

| | | Sample 1 | | | | | Sample 2 | | | | | Mean recovery (%) | SD (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Measured concentration | | | | | Measured concentration | | | | | |
| | Replicate | Spike concentration | Day 1 | Day 2 | Mean | Recovery (%) | Spike concentration | Day 1 | Day 2 | Mean | Recovery (%) | | |
| Sample | 1 | 0 | 119.31 | 247.25 | | | 0 | 88.11 | 55.71 | | | 98.1 | 8.2 |
| | 2 | | 157.16 | 141.29 | 177.1 | | | 50.24 | 57.80 | 63.7 | | | |
| | 3 | | 165.89 | 231.92 | | | | 61.79 | 68.61 | | | | |
| Sample + Spike 1 | 1 | 506.655 | 646.83 | 685.59 | | | 116.42 | 169.07 | 176.07 | | | | |
| | 2 | | 619.61 | 734.63 | 655.19 | 94.36 | | 163.24 | 165.82 | 166.12 | 87.96 | | |
| | 3 | | 608.12 | 636.38 | | | | 171.77 | 150.72 | | | | |
| Sample + Spike 2 | 1 | 101.331 | 237.84 | 370.17 | | | 223.88 | 255.12 | 281.61 | | | | |
| | 2 | | 241.17 | 257.53 | 273.91 | 95.50 | | | 263.44 | 277.61 | 95.54 | | |
| | 3 | | 219.22 | 317.51 | | | | 307.45 | 280.46 | | | | |
| Sample + Spike 3 | 1 | 50.6655 | 201.94 | 336.48 | | | 805.95 | 945.11 | 946.63 | | | | |
| | 2 | | 219.36 | 268.93 | 233.49 | 111.23 | | 930.08 | 819.24 | 902.46 | 104.07 | | |
| | 3 | | 179.09 | 195.16 | | | | 841.79 | 931.88 | | | | |

**Accuracy using Spike Recovery**

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: Aromatic amines in human urine.
Method #: 2020.02
Matrix: **Urine**
Units: pg/mL
Analyte: **26DM**

| | | Sample 1 | | | | | Sample 2 | | | | | Mean recovery (%) | SD (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Measured concentration | | | | | Measured concentration | | | | | |
| | Replicate | Spike concentration | Day 1 | Day 2 | Mean | Recovery (%) | Spike concentration | Day 1 | Day 2 | Mean | Recovery (%) | | |
| Sample | 1 | 0 | 7.68 | 7.57 | | | 0 | 3.18 | 3.91 | | | 97.7 | 5.7 |
| | 2 | | 9.01 | 7.04 | 8.7 | | | 3.07 | 4.37 | 4.2 | | | |
| | 3 | | 10.41 | 10.27 | | | | 3.75 | 6.99 | | | | |
| Sample + Spike 1 | 1 | 55.71 | 58.00 | 54.56 | | | 121.29 | 125.61 | 103.56 | | | | |
| | 2 | | 60.70 | 58.24 | 58.02 | 88.60 | | 135.19 | 115.45 | 121.40 | 96.62 | | |
| | 3 | | 59.91 | 56.71 | | | | 138.18 | 110.43 | | | | |
| Sample + Spike 2 | 1 | 107.14 | 113.68 | 102.76 | | | 144.1 | 174.26 | 147.63 | | | | |
| | 2 | | | 114.42 | 109.70 | 94.31 | | 153.02 | 147.44 | 154.11 | 104.03 | | |
| | 3 | | 116.12 | 101.53 | | | | 147.67 | 154.67 | | | | |
| Sample + Spike 3 | 1 | 385.72 | 402.13 | 383.81 | | | 416.19 | 432.54 | 429.72 | | | | |
| | 2 | | 408.27 | 394.23 | 403.50 | 102.36 | | 435.00 | 425.08 | 420.74 | 100.08 | | |
| | 3 | | 432.34 | 400.25 | | | | 402.57 | 399.56 | | | | |

## Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: Aromatic amines in human urine.
Method #: 2020.02
Matrix: **Urine**
Units: pg/mL
Analyte: **OANS**

| | | Sample 1 | | | | | Sample 2 | | | | | Mean recovery (%) | SD (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | Spike concentration | Measured concentration Day 1 | Day 2 | Mean | Recovery (%) | Spike concentration | Measured concentration Day 1 | Day 2 | Mean | Recovery (%) | | |
| Sample | 1 | 0 | 30.20 | 32.66 | 29.8 | | 0 | 75.01 | 71.54 | 72.6 | | 101.0 | 8.1 |
| | 2 | | 31.32 | 26.46 | | | | 73.60 | 72.14 | | | | |
| | 3 | | 28.84 | 29.59 | | | | (128.591) | 70.54 | | | | |
| Sample + Spike 1 | 1 | 58.48 | 89.94 | 89.06 | 90.13 | 103.09 | 50.91 | 117.26 | 111.07 | 116.08 | 85.48 | | |
| | 2 | | 86.49 | 92.78 | | | | 118.44 | 115.38 | | | | |
| | 3 | | 90.06 | 92.45 | | | | 111.25 | 123.08 | | | | |
| Sample + Spike 2 | 1 | 112.46 | 145.02 | 151.76 | 144.82 | 102.24 | 101.81 | 179.09 | 168.54 | 175.68 | 101.28 | | |
| | 2 | | 155.43 | 132.27 | | | | 178.11 | 178.72 | | | | |
| | 3 | | 153.45 | 131.02 | | | | 172.73 | 176.89 | | | | |
| Sample + Spike 3 | 1 | 404.86 | 492.42 | 414.61 | 458.08 | 105.77 | 203.62 | 284.22 | (498.286) | 293.31 | 108.41 | | |
| | 2 | | 481.03 | 429.40 | | | | 293.82 | 294.48 | | | | |
| | 3 | | 495.74 | 435.30 | | | | 300.42 | 293.63 | | | | |

## Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: Aromatic amines in human urine.
Method #: 2020.02
Matrix: **Urine**
Units: pg/mL
Analyte: **1AMN**

| | | Sample 1 | | | | | Sample 2 | | | | | Mean recovery (%) | SD (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate | Spike concentration | Measured concentration Day 1 | Day 2 | Mean | Recovery (%) | Spike concentration | Measured concentration Day 1 | Day 2 | Mean | Recovery (%) | | |
| Sample | 1 | 0 | 1.59 | 2.27 | 1.6 | | 0 | 1.30 | 3.21 | 2.2 | | 92.2 | 5.5 |
| | 2 | | 1.35 | 1.22 | | | | 1.32 | 2.97 | | | | |
| | 3 | | 1.70 | 1.64 | | | | 1.17 | 3.02 | | | | |
| Sample + Spike 1 | 1 | 110.76 | 99.27 | 122.08 | 109.8 | 97.63 | 52.88 | 47.47 | 46.73 | 47.3 | 85.45 | | |
| | 2 | | 97.10 | 119.18 | | | | 50.21 | 42.79 | | | | |
| | 3 | | 102.32 | 118.64 | | | | 49.84 | 47.05 | | | | |
| Sample + Spike 2 | 1 | 106.61 | 90.88 | 99.04 | 96.1 | 88.65 | 101.7 | 94.07 | 96.97 | 93.9 | 90.25 | | |
| | 2 | | 100.82 | 84.24 | | | | 94.26 | 94.86 | | | | |
| | 3 | | 95.71 | 106.11 | | | | 98.75 | 84.78 | | | | |
| Sample + Spike 3 | 1 | 458.1 | 421.48 | 501.63 | 459 | 99.86 | 366.11 | 326.39 | 327.79 | 337 | 91.51 | | |
| | 2 | | 422.24 | 500.44 | | | | 338.34 | 314.35 | | | | |
| | 3 | | 421.61 | 487.14 | | | | 352.28 | 363.91 | | | | |

## Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: Aromatic amines in human urine.
Method #: 2020.02
Matrix: **Urine**
Units: pg/mL
Analyte: **2AMN**

| | | Sample 1 | | | | | Sample 2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Measured concentration | | | | | Measured concentration | | | |
| | Replicate | Spike concentration | Day 1 | Day 2 | Mean | Recovery (%) | Spike concentration | Day 1 | Day 2 | Mean | Recovery (%) |
| Sample | 1 | 0 | 2.51 | 3.75 | 2.51 | | 0 | 1.72 | 2.30 | 1.83 | |
| | 2 | | 2.54 | 1.60 | | | | 1.61 | 1.74 | | |
| | 3 | | 2.16 | 2.51 | | | | 1.86 | 1.75 | | |
| Sample + Spike 1 | 1 | 489.07 | 508.44 | 543.22 | 528.36 | 107.52 | 111.68 | 116.07 | 117.59 | 113.31 | 99.82 |
| | 2 | | 511.95 | 546.92 | | | | 111.07 | 117.76 | | |
| | 3 | | 520.32 | 539.28 | | | | 102.76 | 114.61 | | |
| Sample + Spike 2 | 1 | 146.721 | 161.90 | 164.00 | 162.96 | 109.36 | 99.14 | 99.39 | 98.16 | 98.19 | 97.19 |
| | 2 | | 153.25 | 168.88 | | | | 98.24 | 99.21 | | |
| | 3 | | 157.48 | 172.25 | | | | 99.66 | 94.48 | | |
| Sample + Spike 3 | 1 | 48.907 | 55.95 | 60.69 | 55.96 | 109.30 | 306.55 | 301.70 | 282.44 | 295.67 | 95.85 |
| | 2 | | 51.29 | 57.95 | | | | 306.00 | 292.66 | | |
| | 3 | | 52.17 | 57.74 | | | | 302.21 | 289.03 | | |

| Mean recovery (%) | SD (%) |
|---|---|
| 103.2 | 6.2 |

## Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: Aromatic amines in human urine.
Method #: 2020.02
Matrix: **Urine**
Units: pg/mL
Analyte: **4ABP**

| | | Sample 1 | | | | | Sample 2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Measured concentration | | | | | Measured concentration | | | |
| | Replicate | Spike concentration | Day 1 | Day 2 | Mean | Recovery (%) | Spike concentration | Day 1 | Day 2 | Mean | Recovery (%) |
| Sample | 1 | 0 | 5.84 | 5.83 | 5.28 | | 0 | 4.39 | 1.93 | 2.82 | |
| | 2 | | 5.92 | 3.15 | | | | 4.07 | 1.49 | | |
| | 3 | | 5.74 | 5.22 | | | | 3.47 | 1.57 | | |
| Sample + Spike 1 | 1 | 109.03 | 108.25 | 107.91 | 108.40 | 94.58 | 54.72 | 53.11 | 48.60 | 50.00 | 86.22 |
| | 2 | | 104.73 | 114.45 | | | | 48.89 | 50.80 | | |
| | 3 | | 101.71 | 113.38 | | | | 51.63 | 46.99 | | |
| Sample + Spike 2 | 1 | 109.17 | 108.67 | 110.67 | 109.45 | 95.42 | 105.24 | 104.52 | 90.55 | 97.49 | 89.96 |
| | 2 | | 111.38 | 107.67 | | | | 102.68 | 89.61 | | |
| | 3 | | 105.59 | 112.71 | | | | 107.60 | 89.97 | | |
| Sample + Spike 3 | 1 | 349.32 | 334.07 | 330.11 | 327.13 | 92.13 | 378.85 | 384.06 | 327.53 | 355.81 | 93.17 |
| | 2 | | 340.64 | 321.86 | | | | 362.76 | 327.06 | | |
| | 3 | | 328.93 | 307.14 | | | | 395.87 | 337.57 | | |

| Mean recovery (%) | SD (%) |
|---|---|
| 91.9 | 3.4 |

## Stability

**Freeze and thaw stability** = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: example: five times frozen at -70°C and then thawed (5 freeze-thaw cycles)
**Bench-top stability** = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: example: original samples (not yet prepared for instrument analysis) frozen at -70°C, thawed, left at room temperature for approx. 4 hours before prepared
**Processed sample stability** = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: example: processed samples (ready for instrument analysis) stored in the cool auto-sampler tray at 10°C for 24 hour before reinjection
**Long-term stability** = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: example: QC samples made in May 2015, stored at -70±10°C for 2 years

All stability sample results should be within ±15% of nominal concentration

Method name: Aromatic amines in human urine.
Method #: 2020.02
Matrix: Urine
Units: pg/mL
Analyte: **OTOL**

| Quality material 1 | | A170828 | | A170808 | | A170828 | | ABC170501 |
|---|---|---|---|---|---|---|---|---|
| | Initial measurement | Three freeze-thaw cycles | Initial measurement | Bench-top stability | Initial measurement | Processed sample stability | Initial measurement | Long-term stability |
| Replicate 1 | 224.88 | 201.83 | 295.26 | 280.28 | 283.608 | 236.12 | 161.53 | 160.8 |
| Replicate 2 | 262.30 | 187.39 | 200.69 | 202.34 | 258.932 | 253.09 | 161.53 | 178.8 |
| Replicate 3 | 203.32 | 205.41 | 199.98 | 291.86 | 217.414 | 263.71 | 161.53 | 167.1 |
| Mean | 230.17 | 198.21 | 231.98 | 258.2 | 253.318 | 250.98 | 161.53 | 168.9 |
| % difference from initial measurement | -- | -13.9 | -- | 11.3 | -- | -0.9 | -- | 4.6 |

| Quality material 2 | | A170808 | | A170808 | | A170828 | | ABC170501 |
|---|---|---|---|---|---|---|---|---|
| | Initial measurement | Three freeze-thaw cycles | Initial measurement | Bench-top stability | Initial measurement | Processed sample stability | Initial measurement | Long-term stability |
| Replicate 1 | 622.97 | 542.71 | 622.97 | 459.99 | 590.785 | 564.95 | 492.29 | 488.2 |
| Replicate 2 | 426.25 | 538.93 | 426.25 | 413.58 | 613.187 | 591.12 | 492.29 | 485.5 |
| Replicate 3 | 470.00 | 489.84 | 470.00 | 560.16 | 574.191 | 578.63 | 492.29 | 505.4 |
| Mean | 506.41 | 523.8 | 506.41 | 477.9 | 592.721 | 578.2 | 492.29 | 493.0 |
| % difference from initial measurement | -- | 3.4 | -- | -5.6 | -- | -2.4 | -- | 0.2 |

## Stability

**Freeze and thaw stability** = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: example: five times frozen at -70°C and then thawed (5 freeze-thaw cycles)
**Bench-top stability** = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: example: original samples (not yet prepared for instrument analysis) frozen at -70°C, thawed, left at room temperature for approx. 4 hours before prepared
**Processed sample stability** = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: example: processed samples (ready for instrument analysis) stored in the cool auto-sampler tray at 10°C for 24 hour before reinjection
**Long-term stability** = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: example: QC samples made in May 2015, stored at -70±10°C for 2 years

All stability sample results should be within ±15% of nominal concentration

Method name: Aromatic amines in human urine.
Method #: 2020.02
Matrix: Urine
Units: pg/mL
Analyte: **26DM**

| Quality material 1 | | A170828 | | A170808 | | A170828 | | ABC170501 |
|---|---|---|---|---|---|---|---|---|
| | Initial measurement | Three freeze-thaw cycles | Initial measurement | Bench-top stability | Initial measurement | Processed sample stability | Initial measurement | Long-term stability |
| Replicate 1 | 174.26 | 179.97 | 174.26 | 175.36 | 125.611 | 126.28 | 119.27 | 103.6 |
| Replicate 2 | 153.02 | 146.80 | 153.02 | 147.34 | 124.631 | 118.98 | 119.27 | 115.4 |
| Replicate 3 | 147.67 | 147.42 | 147.67 | 155.25 | 120.34 | 131.98 | 119.27 | 110.4 |
| Mean | 158.32 | 158.06 | 158.32 | 159.3 | 123.53 | 125.75 | 119.27 | 109.8 |
| % difference from initial measurement | -- | -0.2 | -- | 0.6 | -- | 1.8 | -- | -7.9 |

| Quality material 2 | | A170808 | | A170808 | | A170828 | | ABC170501 |
|---|---|---|---|---|---|---|---|---|
| | Initial measurement | Three freeze-thaw cycles | Initial measurement | Bench-top stability | Initial measurement | Processed sample stability | Initial measurement | Long-term stability |
| Replicate 1 | 459.57 | 399.25 | 459.57 | 429.72 | 525.347 | 487.59 | 503.6 | 514.9 |
| Replicate 2 | 454.49 | 418.56 | 454.49 | 425.08 | 531.809 | 510.95 | 503.6 | 477.4 |
| Replicate 3 | 457.57 | 412.54 | 457.57 | 399.56 | 500.118 | 471.89 | 503.6 | 485.3 |
| Mean | 457.21 | 410.1 | 457.21 | 418.1 | 519.09 | 490.1 | 503.6 | 492.6 |
| % difference from initial measurement | -- | -10.3 | -- | -8.6 | -- | -5.6 | -- | -2.2 |

## Stability

**Freeze and thaw stability** = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: example: five times frozen at -70°C and then thawed (5 freeze-thaw cycles)
**Bench-top stability** = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: example: original samples (not yet prepared for instrument analysis) frozen at -70°C, thawed, left at room temperature for approx. 4 hours before prepared
**Processed sample stability** = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: example: processed samples (ready for instrument analysis) stored in the cool auto-sampler tray at 10°C for 24 hour before reinjection
**Long-term stability** = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: example: QC samples made in May 2015, stored at -70±10°C for 2 years

All stability sample results should be within ±15% of nominal concentration

Method name: Aromatic amines in human urine.
Method #: 2020.02
Matrix: Urine
Units: pg/mL
Analyte: **OANS**

| Quality material 1 | | A170828 | | A170808 | | A170828 | | ABC170501 |
|---|---|---|---|---|---|---|---|---|
| | Initial measurement | Three freeze-thaw cycles | Initial measurement | Bench-top stability | Initial measurement | Processed sample stability | Initial measurement | Long-term stability |
| Replicate 1 | 151.21 | 140.70 | 151.21 | 148.89 | 155.149 | 161.82 | 155.14 | 143.3 |
| Replicate 2 | 143.32 | 145.12 | 143.32 | 136.86 | 156.647 | 161.11 | 155.14 | 149.0 |
| Replicate 3 | 151.22 | 147.58 | 151.22 | 142.93 | 160.037 | 162.48 | 155.14 | 151.8 |
| Mean | 148.59 | 144.47 | 148.59 | 142.9 | 157.28 | 161.80 | 155.14 | 148.0 |
| % difference from initial measurement | -- | -2.8 | -- | -3.8 | -- | 2.9 | -- | -4.6 |

| Quality material 2 | | A170808 | | A170808 | | A170828 | | ABC170501 |
|---|---|---|---|---|---|---|---|---|
| | Initial measurement | Three freeze-thaw cycles | Initial measurement | Bench-top stability | Initial measurement | Processed sample stability | Initial measurement | Long-term stability |
| Replicate 1 | 427.06 | 377.25 | 427.06 | 399.84 | 475.003 | 491.15 | 502.18 | 511.7 |
| Replicate 2 | 402.21 | 388.93 | 402.21 | 375.67 | 501.076 | 483.17 | 502.18 | 496.5 |
| Replicate 3 | 404.62 | 380.46 | 404.62 | 376.16 | 490.281 | 462.83 | 502.18 | 487.3 |
| Mean | 411.30 | 382.2 | 411.30 | 383.9 | 488.79 | 479.05 | 502.18 | 498.5 |
| % difference from initial measurement | -- | -7.1 | -- | -6.7 | -- | -2.0 | -- | -0.7 |

## Stability

**Freeze and thaw stability** = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: example: five times frozen at -70°C and then thawed (5 freeze-thaw cycles)
**Bench-top stability** = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: example: original samples (not yet prepared for instrument analysis) frozen at -70°C, thawed, left at room temperature for approx. 4 hours before prepared
**Processed sample stability** = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: example: processed samples (ready for instrument analysis) stored in the cool auto-sampler tray at 10°C for 24 hour before reinjection
**Long-term stability** = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: example: QC samples made in May 2015, stored at -70±10°C for 2 years

All stability sample results should be within ±15% of nominal concentration

Method name: Aromatic amines in human urine.
Method #: 2020.02
Matrix: Urine
Units: pg/mL
Analyte: **1AMN**

| Quality material 1 | | A170828 | | A170808 | | A170828 | | ABC170501 |
|---|---|---|---|---|---|---|---|---|
| | Initial measurement | Three freeze-thaw cycles | Initial measurement | Bench-top stability | Initial measurement | Processed sample stability | Initial measurement | Long-term stability |
| Replicate 1 | 90.88 | 88.94 | 90.88 | 89.68 | 107.104 | 112.18 | 110.76 | 122.1 |
| Replicate 2 | 100.82 | 92.49 | 100.82 | 98.38 | 109.298 | 101.80 | 110.76 | 119.2 |
| Replicate 3 | 95.71 | 90.51 | 95.71 | 99.67 | 101.758 | 100.82 | 110.76 | 118.6 |
| Mean | 95.80 | 90.65 | 95.80 | 95.9 | 106.05 | 104.93 | 110.76 | 120.0 |
| % difference from initial measurement | -- | -5.4 | -- | 0.1 | -- | -1.1 | -- | 8.3 |

| Quality material 2 | | A170808 | | A170808 | | A170828 | | ABC170501 |
|---|---|---|---|---|---|---|---|---|
| | Initial measurement | Three freeze-thaw cycles | Initial measurement | Bench-top stability | Initial measurement | Processed sample stability | Initial measurement | Long-term stability |
| Replicate 1 | 269.21 | 261.05 | 269.21 | 288.82 | 444.881 | 429.71 | 454.24 | 501.6 |
| Replicate 2 | 300.47 | 273.78 | 300.47 | 311.55 | 434.998 | 443.18 | 454.24 | 500.4 |
| Replicate 3 | 329.36 | 285.60 | 329.36 | 296.55 | 424.66 | 419.29 | 454.24 | 487.1 |
| Mean | 299.68 | 273.5 | 299.68 | 299.0 | 434.85 | 430.7 | 454.24 | 496.4 |
| % difference from initial measurement | -- | -8.7 | -- | -0.2 | -- | -0.9 | -- | 9.3 |

## Stability

**Freeze and thaw stability** = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: example: five times frozen at -70°C and then thawed (5 freeze-thaw cycles)
**Bench-top stability** = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: example: original samples (not yet prepared for instrument analysis) frozen at -70°C, thawed, left at room temperature for approx. 4 hours before prepared
**Processed sample stability** = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: example: processed samples (ready for instrument analysis) stored in the cool auto-sampler tray at 10°C for 24 hour before reinjection
**Long-term stability** = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: example: QC samples made in May 2015, stored at -70±10°C for 2 years

All stability sample results should be within ±15% of nominal concentration

Method name: Aromatic amines in human urine.
Method #: 2020.02
Matrix: Urine
Units: pg/mL
Analyte: **2AMN**

| Quality material 1 | | A170828 | | A170808 | | A170828 | | ABC170501 |
|---|---|---|---|---|---|---|---|---|
| | Initial measurement | Three freeze-thaw cycles | Initial measurement | Bench-top stability | Initial measurement | Processed sample stability | Initial measurement | Long-term stability |
| Replicate 1 | 99.39 | 99.27 | 99.39 | 96.55 | 116.067 | 113.28 | 111.63 | 117.6 |
| Replicate 2 | 98.24 | 97.77 | 98.24 | 92.69 | 114.422 | 114.74 | 111.63 | 117.8 |
| Replicate 3 | 99.66 | 90.82 | 99.66 | 99.27 | 121.123 | 116.57 | 111.63 | 114.6 |
| Mean | 99.10 | 95.95 | 99.10 | 96.2 | 117.20 | 114.87 | 111.63 | 116.7 |
| % difference from initial measurement | -- | -3.2 | -- | -3.0 | -- | -2.0 | -- | 4.5 |

| Quality material 2 | | A170808 | | A170808 | | A170828 | | ABC170501 |
|---|---|---|---|---|---|---|---|---|
| | Initial measurement | Three freeze-thaw cycles | Initial measurement | Bench-top stability | Initial measurement | Processed sample stability | Initial measurement | Long-term stability |
| Replicate 1 | 318.76 | 255.50 | 318.76 | 282.44 | 475.08 | 466.81 | 477.84 | 521.6 |
| Replicate 2 | 316.33 | 264.26 | 316.33 | 292.66 | 473.418 | 489.88 | 477.84 | 503.4 |
| Replicate 3 | 315.29 | 284.68 | 315.29 | 289.03 | 481.723 | 466.57 | 477.84 | 509.5 |
| Mean | 316.79 | 268.14 | 316.79 | 288.0 | 476.74 | 474.42 | 477.84 | 511.5 |
| % difference from initial measurement | -- | -15.4 | -- | -9.1 | -- | -0.5 | -- | 7.0 |

## Stability

**Freeze and thaw stability** = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: example: five times frozen at -70°C and then thawed (5 freeze-thaw cycles)
**Bench-top stability** = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: example: original samples (not yet prepared for instrument analysis) frozen at -70°C, thawed, left at room temperature for approx. 4 hours before prepared
**Processed sample stability** = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: example: processed samples (ready for instrument analysis) stored in the cool auto-sampler tray at 10°C for 24 hour before reinjection
**Long-term stability** = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: example: QC samples made in May 2015, stored at -70±10°C for 2 years

All stability sample results should be within ±15% of nominal concentration

Method name: Aromatic amines in human urine.
Method #: 2020.02
Matrix: Urine
Units: pg/mL
Analyte: **4ABP**

| Quality material 1 | | A170808 | | A170808 | | A170828 | | ABC170501 |
|---|---|---|---|---|---|---|---|---|
| | Initial measurement | Three freeze-thaw cycles | Initial measurement | Bench-top stability | Initial measurement | Processed sample stability | Initial measurement | Long-term stability |
| Replicate 1 | 108.67 | 98.89 | 99.04 | 105.81 | 108.252 | 110.65 | 110.29 | 107.9 |
| Replicate 2 | 111.38 | 103.30 | 84.24 | 102.69 | 105.265 | 109.31 | 110.29 | 114.4 |
| Replicate 3 | 105.59 | 102.62 | 106.11 | 106.50 | 111.863 | 108.81 | 110.29 | 113.4 |
| Mean | 108.55 | 101.60 | 96.46 | 105.0 | 108.46 | 109.59 | 110.29 | 111.9 |
| % difference from initial measurement | -- | -6.4 | -- | 8.8 | -- | 1.0 | -- | 1.5 |

| Quality material 2 | | A170808 | | A170808 | | A170828 | | ABC170501 |
|---|---|---|---|---|---|---|---|---|
| | Initial measurement | Three freeze-thaw cycles | Initial measurement | Bench-top stability | Initial measurement | Processed sample stability | Initial measurement | Long-term stability |
| Replicate 1 | 347.03 | 300.35 | 347.03 | 330.11 | 426.174 | 430.10 | 441.33 | 473.9 |
| Replicate 2 | 336.31 | 314.18 | 336.31 | 321.86 | 423.264 | 430.90 | 441.33 | 463.3 |
| Replicate 3 | 337.61 | 323.48 | 337.61 | 307.14 | 435.255 | 437.58 | 441.33 | 465.2 |
| Mean | 336.96 | 312.7 | 336.96 | 319.7 | 428.23 | 432.86 | 441.33 | 467.5 |
| % difference from initial measurement | -- | -7.2 | -- | -5.1 | -- | 1.1 | -- | 5.9 |

## Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name: Aromatic amines in human urine.
Method #: 2020.02
Matrix: Urine
Units: pg/mL
Analyte: **OTOL**

**Quality material 1**

| Run | Result 1 | Result 2 | Mean | SS 1 | SS 2 | 2*mean^2 |
|---|---|---|---|---|---|---|
| 1 | 196.4439198 | 169.26 | 182.85 | 184.7413737 | 184.7413737 | 66869.67847 |
| 2 | 191.0568 | 205.456 | 198.26 | 51.83424016 | 51.83424016 | 78611.20028 |
| 3 | 186.75 | 162.061 | 174.41 | 152.3866803 | 152.3866803 | 60834.55686 |
| 4 | 184.422 | 171.538 | 177.98 | 41.499364 | 41.499364 | 63353.7608 |
| 5 | 169.118 | 165.294 | 167.21 | 3.655744 | 3.655744 | 55915.69287 |
| 6 | 211.86 | 198.473 | 205.17 | 44.80294225 | 44.80294225 | 84186.58544 |
| 7 | 153.917 | 182.033 | 167.98 | 197.627364 | 197.627364 | 56431.20125 |
| 8 | 218.799 | 172.885 | 195.84 | 527.023849 | 527.023849 | 76708.17793 |
| 9 | 163.18 | 178.374 | 170.78 | 57.714409 | 57.714409 | 58329.56746 |
| 10 | 221.08 | 192.69 | 206.88 | 201.441249 | 201.441249 | 85601.15138 |

**Grand sum** 3694.68672 **Grand mean** 184.734336

| | Sum squares | Mean Sq Error | Std Dev | Rel Std Dev (%) |
|---|---|---|---|---|
| **Within Run** | 2925.454431 | 292.5454431 | 17.10395987 | 9.26 |
| **Between Run** | 4306.074878 | 478.4527642 | 9.641247875 | 5.22 |
| **Total** | 7231.529309 | | 19.63413109 | **10.63** |

**Quality material 2**

| Run | Result 1 | Result 2 | Mean | SS 1 | SS 2 | 2*mean^2 |
|---|---|---|---|---|---|---|
| 1 | 414.03 | 438.82 | 426.42 | 153.6473889 | 153.6473889 | 363668.9566 |
| 2 | 533.46 | 412.06 | 472.76 | 3684.589324 | 3684.589324 | 447011.2555 |
| 3 | 460.03 | 423.46 | 441.75 | 334.4692323 | 334.4692323 | 390278.1735 |
| 4 | 483.59 | 386.14 | 434.87 | 2374.028176 | 2374.028176 | 378218.6154 |
| 5 | 396.21 | 455.81 | 426.01 | 888.0102003 | 888.0102002 | 362975.0044 |
| 6 | 418.56 | 420.17 | 419.37 | 0.64561225 | 0.64561225 | 351736.5226 |
| 7 | 428.21 | 433.45 | 430.83 | 6.8644 | 6.8644 | 371232.4244 |
| 8 | 349.81 | 450.52 | 400.17 | 2535.525316 | 2535.525316 | 320267.2558 |
| 9 | 419.93 | 451.52 | 435.72 | 249.450436 | 249.450436 | 379710.8084 |
| 10 | 427.87 | 408.14 | 418.01 | 97.318225 | 97.318225 | 349456.3601 |

**Grand sum** 8611.80972 **Grand mean** 430.590486

| | Sum squares | Mean Sq Error | Std Dev | Rel Std Dev |
|---|---|---|---|---|
| **Within Run** | 20649.09662 | 2064.909662 | 45.44127708 | 10.55 |
| **Between Run** | 6392.044413 | 710.227157 | 0 | 0.00 |
| **Total** | 27041.14103 | | 45.44127708 | **10.55** |

## Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name: Aromatic amines in human urine.
Method #: 2020.02
Matrix: Urine
Units: pg/mL
Analyte: **26DM**

**Quality material 1**

| Run | Result 1 | Result 2 | Mean | SS 1 | SS 2 | 2*mean^2 |
|---|---|---|---|---|---|---|
| 1 | 115.13 | 117.66 | 116.40 | 1.600225 | 1.600225 | 27095.59205 |
| 2 | 119.99 | 120.1 | 120.05 | 0.003025 | 0.003025 | 28821.60405 |
| 3 | 139.04 | 154.76 | 146.90 | 61.7796 | 61.7796 | 43159.22 |
| 4 | 117.76 | 122.1 | 119.93 | 4.7089 | 4.7089 | 28766.4098 |
| 5 | 128.35 | 115.76 | 122.06 | 39.627025 | 39.627025 | 29794.84605 |
| 6 | 125.28 | 125.64 | 125.46 | 0.0324 | 0.0324 | 31480.4232 |
| 7 | 127.55 | 125.8 | 126.68 | 0.765625 | 0.765625 | 32093.11125 |
| 8 | 127.77 | 120.86 | 124.32 | 11.937025 | 11.937025 | 30908.43845 |
| 9 | 150.91 | 183.24 | 167.08 | 261.307225 | 261.307225 | 55828.11125 |
| 10 | 107.8 | 92.77 | 100.29 | 56.475225 | 56.475225 | 20114.16245 |

**Grand sum** 2538.27 **Grand mean** 126.9135

| | Sum squares | Mean Sq Error | Std Dev | Rel Std Dev (%) |
|---|---|---|---|---|
| **Within Run** | 876.47255 | 87.647255 | 9.362011269 | 7.38 |
| **Between Run** | 5921.188905 | 657.9098783 | 16.88583168 | 13.30 |
| **Total** | 6797.661455 | | 19.30747437 | **15.21** |

**Quality material 2**

| Run | Result 1 | Result 2 | Mean | SS 1 | SS 2 | 2*mean^2 |
|---|---|---|---|---|---|---|
| 1 | 503.91 | 533.86 | 518.89 | 224.250625 | 224.250625 | 538483.2865 |
| 2 | 504.78 | 490.64 | 497.71 | 49.9849 | 49.9849 | 495430.4882 |
| 3 | 466.59 | 480.5 | 473.55 | 48.372025 | 48.372025 | 448489.7341 |
| 4 | 505.63 | 493.99 | 499.81 | 33.8724 | 33.8724 | 499620.0722 |
| 5 | 514.46 | 506.1 | 510.28 | 17.4724 | 17.4724 | 520771.3568 |
| 6 | 499.07 | 501.57 | 500.32 | 1.5625 | 1.5625 | 500640.2048 |
| 7 | 535.09 | 495.02 | 515.06 | 401.401225 | 401.401225 | 530563.3061 |
| 8 | 538.91 | 512.1 | 525.51 | 179.694025 | 179.694025 | 552311.0101 |
| 9 | 502.36 | 476.66 | 489.51 | 165.1225 | 165.1225 | 479240.0802 |
| 10 | 457.72 | 484.34 | 471.03 | 177.1561 | 177.1561 | 443738.5218 |

**Grand sum** 10003.3 **Grand mean** 500.165

| | Sum squares | Mean Sq Error | Std Dev | Rel Std Dev |
|---|---|---|---|---|
| **Within Run** | 2597.7774 | 259.77774 | 16.11762203 | 3.22 |
| **Between Run** | 5987.5161 | 665.2795667 | 14.23906294 | 2.85 |
| **Total** | 8585.2935 | | 21.50647933 | **4.30** |

## Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name: Aromatic amines in human urine.
Method #: 2020.02
Matrix: Urine
Units: pg/mL
Analyte: **OANS**

**Quality material 1**

| Run | Result 1 | Result 2 | Mean | SS 1 | SS 2 | 2*mean^2 |
|---|---|---|---|---|---|---|
| 1 | 153.94 | 149.2 | 151.57 | 5.6169 | 5.6169 | 45946.9298 |
| 2 | 149.31 | 151.24 | 150.28 | 0.931225 | 0.931225 | 45165.15125 |
| 3 | 161.19 | 149.34 | 155.27 | 35.105625 | 35.105625 | 48214.44045 |
| 4 | 146.67 | 151.04 | 148.86 | 4.774225 | 4.774225 | 44315.62205 |
| 5 | 151.97 | 168.44 | 160.21 | 67.815225 | 67.815225 | 51331.28405 |
| 6 | 141.68 | 149.28 | 145.48 | 14.44 | 14.44 | 42328.8608 |
| 7 | 152.21 | 151.93 | 152.07 | 0.0196 | 0.0196 | 46250.5698 |
| 8 | 146.29 | 151.07 | 148.68 | 5.7121 | 5.7121 | 44211.4848 |
| 9 | 149.34 | 156.12 | 152.73 | 11.4921 | 11.4921 | 46652.9058 |
| 10 | 150.72 | 148.4 | 149.56 | 1.3456 | 1.3456 | 44736.3872 |

**Grand sum** 3029.38 **Grand mean** 151.469

| | Sum squares | Mean Sq Error | Std Dev | Rel Std Dev (%) |
|---|---|---|---|---|
| **Within Run** | 294.5052 | 29.45052 | 5.426833331 | 3.58 |
| **Between Run** | 296.47678 | 32.94186444 | 1.321238897 | 0.87 |
| **Total** | 590.98198 | | 5.585355156 | **3.69** |

**Quality material 2**

| Run | Result 1 | Result 2 | Mean | SS 1 | SS 2 | 2*mean^2 |
|---|---|---|---|---|---|---|
| 1 | 516.13 | 476.84 | 496.49 | 385.926025 | 385.926025 | 492994.7105 |
| 2 | 492.49 | 478.06 | 485.28 | 52.056225 | 52.056225 | 470983.6513 |
| 3 | 537.93 | 531.95 | 534.94 | 8.9401 | 8.9401 | 572321.6072 |
| 4 | 486.47 | 452.17 | 469.32 | 294.1225 | 294.1225 | 440522.5248 |
| 5 | 526.86 | 513.84 | 520.35 | 42.3801 | 42.3801 | 541528.245 |
| 6 | 460.06 | 454.75 | 457.41 | 7.049025 | 7.049025 | 418438.6681 |
| 7 | 472.39 | 484.93 | 478.66 | 39.3129 | 39.3129 | 458230.7912 |
| 8 | 487.53 | 542.9 | 515.22 | 766.459225 | 766.459225 | 530892.9925 |
| 9 | 487.01 | 472.78 | 479.90 | 50.623225 | 50.623225 | 460598.4221 |
| 10 | 470.95 | 483.61 | 477.28 | 40.0689 | 40.0689 | 455592.3968 |

**Grand sum** 9829.65 **Grand mean** 491.4825

| | Sum squares | Mean Sq Error | Std Dev | Rel Std Dev |
|---|---|---|---|---|
| **Within Run** | 3373.87645 | 337.387645 | 18.3681149 | 3.74 |
| **Between Run** | 11003.05312 | 1222.561458 | 21.03774956 | 4.28 |
| **Total** | 14376.92957 | | 27.92802449 | **5.68** |

## Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name: Aromatic amines in human urine.
Method #: 2020.02
Matrix: Urine
Units: pg/mL
Analyte: **1AMN**

**Quality material 1**

| Run | Result 1 | Result 2 | Mean | SS 1 | SS 2 | 2*mean^2 |
|---|---|---|---|---|---|---|
| 1 | 94.47 | 109.31 | 101.89 | 55.0564 | 55.0564 | 20763.1442 |
| 2 | 102.74 | 106.9 | 104.82 | 4.3264 | 4.3264 | 21974.4648 |
| 3 | 110.52 | 118.28 | 114.40 | 15.0544 | 15.0544 | 26174.72 |
| 4 | 102.53 | 99.92 | 101.23 | 1.703025 | 1.703025 | 20493.00125 |
| 5 | 104.92 | 119.3 | 112.11 | 51.6961 | 51.6961 | 25137.3042 |
| 6 | 105.5 | 106.63 | 106.07 | 0.319225 | 0.319225 | 22499.56845 |
| 7 | 112.27 | 115.53 | 113.90 | 2.6569 | 2.6569 | 25946.42 |
| 8 | 100.44 | 95.9 | 98.17 | 5.1529 | 5.1529 | 19274.6978 |
| 9 | 105.4 | 116.43 | 110.92 | 30.415225 | 30.415225 | 24604.27445 |
| 10 | 96.16 | 109.74 | 102.95 | 46.1041 | 46.1041 | 21197.405 |

**Grand sum** 2132.89 **Grand mean** 106.6445

| | Sum squares | Mean Sq Error | Std Dev | Rel Std Dev (%) |
|---|---|---|---|---|
| **Within Run** | 424.96935 | 42.496935 | 6.518967326 | 6.11 |
| **Between Run** | 604.012545 | 67.112505 | 3.508245288 | 3.29 |
| **Total** | 1028.981895 | | 7.403021005 | **6.94** |

**Quality material 2**

| Run | Result 1 | Result 2 | Mean | SS 1 | SS 2 | 2*mean^2 |
|---|---|---|---|---|---|---|
| 1 | 426.58 | 437.99 | 432.29 | 32.547025 | 32.547025 | 373740.6425 |
| 2 | 409.8 | 437.26 | 423.53 | 188.5129 | 188.5129 | 358755.3218 |
| 3 | 470.74 | 472.25 | 471.50 | 0.570025 | 0.570025 | 444615.0701 |
| 4 | 459.56 | 476.12 | 467.84 | 68.5584 | 68.5584 | 437748.5312 |
| 5 | 449.14 | 426.81 | 437.98 | 124.657225 | 124.657225 | 383644.2013 |
| 6 | 453.96 | 455.61 | 454.79 | 0.680625 | 0.680625 | 413658.7925 |
| 7 | 434.8 | 424.29 | 429.55 | 27.615025 | 27.615025 | 369017.8141 |
| 8 | 423.27 | 447.88 | 435.58 | 151.413025 | 151.413025 | 379451.1613 |
| 9 | 403.41 | 439.24 | 421.33 | 320.947225 | 320.947225 | 355029.5113 |
| 10 | 421.06 | 458.47 | 439.77 | 349.877025 | 349.877025 | 386786.5105 |

**Grand sum** 8828.24 **Grand mean** 441.412

| | Sum squares | Mean Sq Error | Std Dev | Rel Std Dev |
|---|---|---|---|---|
| **Within Run** | 2530.757 | 253.0757 | 15.90835315 | 3.60 |
| **Between Run** | 5556.48132 | 617.3868133 | 13.49650165 | 3.06 |
| **Total** | 8087.23832 | | 20.86219683 | **4.73** |

## Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name: Aromatic amines in human urine.
Method #: 2020.02
Matrix: Urine
Units: pg/mL
Analyte: **2AMN**

**Quality material 1**

| Run | Result 1 | Result 2 | Mean | SS 1 | SS 2 | 2*mean^2 |
|---|---|---|---|---|---|---|
| 1 | 103.38 | 97.3 | 100.34 | 9.2416 | 9.2416 | 20136.2312 |
| 2 | 106.58 | 106.85 | 106.72 | 0.018225 | 0.018225 | 22776.18245 |
| 3 | 115.18 | 116.22 | 115.70 | 0.2704 | 0.2704 | 26772.98 |
| 4 | 109.14 | 113.59 | 111.37 | 4.950625 | 4.950625 | 24804.32645 |
| 5 | 107.33 | 104.28 | 105.81 | 2.325625 | 2.325625 | 22389.39605 |
| 6 | 110.37 | 111 | 110.69 | 0.099225 | 0.099225 | 24502.33845 |
| 7 | 97.67 | 107.2 | 102.44 | 22.705225 | 22.705225 | 20985.85845 |
| 8 | 104.7 | 106.09 | 105.40 | 0.483025 | 0.483025 | 22216.21205 |
| 9 | 99.92 | 103.07 | 101.50 | 2.480625 | 2.480625 | 20602.47005 |
| 10 | 112.4 | 113.87 | 113.14 | 0.540225 | 0.540225 | 25599.05645 |

**Grand sum** 2146.14 **Grand mean** 107.307

| | Sum squares | Mean Sq Error | Std Dev | Rel Std Dev (%) |
|---|---|---|---|---|
| **Within Run** | 86.2296 | 8.62296 | 2.936487698 | 2.74 |
| **Between Run** | 489.20662 | 54.35629111 | 4.781910241 | 4.46 |
| **Total** | 575.43622 | | 5.611561775 | **5.23** |

**Quality material 2**

| Run | Result 1 | Result 2 | Mean | SS 1 | SS 2 | 2*mean^2 |
|---|---|---|---|---|---|---|
| 1 | 428.74 | 427.92 | 428.33 | 0.1681 | 0.1681 | 366933.1778 |
| 2 | 443.6 | 450.4 | 447.00 | 11.56 | 11.56 | 399618 |
| 3 | 462.64 | 513.27 | 487.96 | 640.849225 | 640.849225 | 476200.1641 |
| 4 | 464.61 | 471.91 | 468.26 | 13.3225 | 13.3225 | 438534.8552 |
| 5 | 463.3 | 457.43 | 460.37 | 8.614225 | 8.614225 | 423871.8665 |
| 6 | 465.14 | 467.8 | 466.47 | 1.7689 | 1.7689 | 435188.5218 |
| 7 | 459.38 | 458.91 | 459.15 | 0.055225 | 0.055225 | 421628.2621 |
| 8 | 450.97 | 484.35 | 467.66 | 278.5561 | 278.5561 | 437411.7512 |
| 9 | 448.34 | 449.88 | 449.11 | 0.5929 | 0.5929 | 403399.5842 |
| 10 | 457.43 | 474.31 | 465.87 | 71.2336 | 71.2336 | 434069.7138 |

**Grand sum** 9200.33 **Grand mean** 460.0165

| | Sum squares | Mean Sq Error | Std Dev | Rel Std Dev |
|---|---|---|---|---|
| **Within Run** | 2053.44155 | 205.344155 | 14.32983444 | 3.12 |
| **Between Run** | 4552.291105 | 505.8101228 | 12.25695655 | 2.66 |
| **Total** | 6605.732655 | | 18.85675314 | **4.10** |

## Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name: Aromatic amines in human urine.
Method #: 2020.02
Matrix: Urine
Units: pg/mL
Analyte: **4ABP**

**Quality material 1**

| Run | Result 1 | Result 2 | Mean | SS 1 | SS 2 | 2*mean^2 |
|---|---|---|---|---|---|---|
| 1 | 100.45 | 99.82 | 100.14 | 0.099225 | 0.099225 | 20054.03645 |
| 2 | 99.82 | 105.58 | 102.70 | 8.2944 | 8.2944 | 21094.58 |
| 3 | 113.99 | 111 | 112.50 | 2.235025 | 2.235025 | 25310.25005 |
| 4 | 108.12 | 111.26 | 109.69 | 2.4649 | 2.4649 | 24063.7922 |
| 5 | 108.33 | 106.14 | 107.24 | 1.199025 | 1.199025 | 22998.69045 |
| 6 | 109.08 | 110.48 | 109.78 | 0.49 | 0.49 | 24103.2968 |
| 7 | 106.7 | 100.53 | 103.62 | 9.517225 | 9.517225 | 21472.13645 |
| 8 | 104.92 | 100.99 | 102.96 | 3.861225 | 3.861225 | 21199.46405 |
| 9 | 105.39 | 108.3 | 106.85 | 2.117025 | 2.117025 | 22831.70805 |
| 10 | 103.96 | 105.95 | 104.96 | 0.990025 | 0.990025 | 22031.10405 |

**Grand sum** 2120.81 **Grand mean** 106.0405

| | Sum squares | Mean Sq Error | Std Dev | Rel Std Dev (%) |
|---|---|---|---|---|
| **Within Run** | 62.53615 | 6.253615 | 2.500722895 | 2.36 |
| **Between Run** | 267.305745 | 29.70063833 | 3.4239614 | 3.23 |
| **Total** | 329.841895 | | 4.239944182 | **4.00** |

**Quality material 2**

| Run | Result 1 | Result 2 | Mean | SS 1 | SS 2 | 2*mean^2 |
|---|---|---|---|---|---|---|
| 1 | 415.54 | 412.6 | 414.07 | 2.1609 | 2.1609 | 342907.9298 |
| 2 | 413.47 | 405.16 | 409.32 | 17.264025 | 17.264025 | 335077.5385 |
| 3 | 475.49 | 461.01 | 468.25 | 52.4176 | 52.4176 | 438516.125 |
| 4 | 436.05 | 423.1 | 429.58 | 41.925625 | 41.925625 | 369069.3613 |
| 5 | 428.65 | 432.55 | 430.60 | 3.8025 | 3.8025 | 370832.72 |
| 6 | 420.59 | 417.79 | 419.19 | 1.96 | 1.96 | 351440.5122 |
| 7 | 435.7 | 433.08 | 434.39 | 1.7161 | 1.7161 | 377389.3442 |
| 8 | 431.05 | 431.24 | 431.15 | 0.009025 | 0.009025 | 371772.0221 |
| 9 | 426.53 | 413.52 | 420.03 | 42.315025 | 42.315025 | 352842.0013 |
| 10 | 429.12 | 419.1 | 424.11 | 25.1001 | 25.1001 | 359738.5842 |

**Grand sum** 8561.34 **Grand mean** 428.067

| | Sum squares | Mean Sq Error | Std Dev | Rel Std Dev |
|---|---|---|---|---|
| **Within Run** | 377.3418 | 37.73418 | 6.142815315 | 1.44 |
| **Between Run** | 4759.00862 | 528.7787356 | 15.66915051 | 3.66 |
| **Total** | 5136.35042 | | 16.83022453 | **3.93** |

## LOD, specificity and fit for intended use

Method name: Aromatic amines in human urine.
Method #: 2020.02
Matrix: Urine
Units: pg/mL

| Analytes | Limit of Detection (LOD) | Interferences successfully checked in at least 50 human samples | Accuracy, precision, LOD, specificity and stability meet performance specifications for intended use |
|---|---|---|---|
| OTOL | 111.22 | yes[1] | yes |
| 26DM | 15.67 | yes[2] | yes |
| OANS | 7.02 | yes[3] | yes |
| 1AMN | 1.29 | yes | yes |
| 2AMN | 2.79 | yes | yes |
| 4ABP | 1.75 | yes | yes |

N=50

[1] interference is observed in Qual channel, approximately 10% in general population

[2] interference is observed in Qual channel, approximately 6% in general population

[3] interference is observed in both Quant and Qual channel, approximately 6% in general population